Plant Growth Hormones

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I. Historical Development and Definitions

The concept of hormones in plants developed from the study of tropisms or curvatures. Growing shoots typically curve toward a source of light (positive phototropism), and away from the earth (negative geotropism), while roots curve toward the earth (positive geotropism), and in some cases away from light (negative phototropism). Curvatures may also occur away from or toward wounds (traumatotropism), electrodes (electrotropism), water (hydrotropism), etc. All these curvatures depend fundamentally on a difference in growth rate between the two sides of a growing organ—the convex side grows faster than the concave.

The careful studies of Charles and Francis Darwin on the geotropism and phototropism of seedlings (72) made it clear that the perception of light and gravity is centered in the tip of the growing organ; thus phototropism of the coleoptile of Phalaris (a grass) was prevented completely by covering the extreme tip with a black paper cap. Nevertheless, the Darwins observed that the curvature in such tropisms is not restricted to the tip but spreads downward to the basal regions. They concluded that some "influence" is "transmitted" from the tip to the basal regions.

Thirty years later Boysen-Jensen (41,42) showed that this influence can cross a discontinuity. He cut off the tips of Avena (oat) coleoptiles and stuck them on again in situ with gelatin. On now illuminating the tips, curvature appeared first in the tip and then also in the base. Evidently the influence which is transmitted must be of a "material nature." This experiment was repeated with numerous variations, refinements, and controls by Paál (239). More important, however, was the following experiment (done with Coix coleoptiles): the tip was cut off and then replaced, not symmetrically, but a little to one side. Without any illumination the plant now curved so that the side in contact with the tip

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1 For a fuller treatment see Went and Thimann (360) Chapter 2, and also Boysen-Jensen (46) Chapter 1.
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was convex. This side, therefore, grew more than the other, and Paál
deduced that in the tip "a substance (or a mixture) is formed and inter-
entially secreted." This substance diffuses into the lower regions and
controls growth there. In normal growth, this substance would be
symmetrically distributed, but curvature would be due to asymmetric
distribution, caused in some way by the light (or gravity).

This conclusion led to experiments on normal, not curved, growth.
Söding (287, 288) showed that indeed the tip controls straight growth of
the part below it. Decapitation slows the growth greatly, though after
some hours there is an acceleration due to "regeneration of the physio-
logical tip" in the apical part of the remaining shoot. This regeneration
was subsequently shown (78, 288) to be due to production of the growth-
promoting substance by the most apical remaining tissue.

Extracts of various tissues mixed with agar and applied to one side of
decapitated coleoptiles (294) gave no evidence of containing a growth
substance, though the technique of these experiments was a valuable
advance. Certain enzyme extracts applied in agar did produce curva-
tures, however (Seubert, 269).

Finally, Went (347, 348) placed cut-off coleoptile tips on agar and
applied this agar to decapitated coleoptiles. This caused curvature,
the side in contact with the agar being convex. Evidently the growth
substance, although it could not be extracted by crushing the tissue,
would "diffuse" from the intact tip into agar. The curvature was shown
to be proportional, within limits, to the amount of growth substance in
the agar, i.e., to the number of the tips placed on each block and the
length of time they had been in contact. This procedure has formed the
basis for the assay method described below, by means of which three
naturally occurring substances of similar growth-promoting action have
been isolated and many aspects of growth physiology have been studied.
The growth hormones have been named "auxins" and this name has
since been applied to the whole group of synthetic substances of similar
activity.

The remaining historical development will be treated in the appro-
priate sections.

Definitions

Considerable confusion in the use of the terms: growth substance,
growth hormone, growth regulator, Wuchsstoff, phytohormone, forma-
tive substance, and auxin has arisen. The following definitions, which
are practical rather than rigid, are put forward to simplify the situation.2

1 Similar, but not identical, definitions have been proposed by van Overbeek (234a)
Auxin. An organic substance which promotes growth (i.e., irreversible increase in volume) along the longitudinal axis, when applied in low concentrations to shoots of plants freed as far as practical from their own inherent growth-promoting substance. Auxins may, and generally do, have other properties, but this one is critical. The definition excludes nutrient salts, and in order to exclude sugar, which unquestionably promotes longitudinal growth, the term “low concentrations” may conveniently be interpreted as “below M/1000.” Most auxins produce clear-cut growth effects at $10^{-8}$ M or even considerably below.¹

Phytohormone. An organic substance produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production, and active in minute amounts. This definition includes those auxins which are of natural occurrence, certain of the vitamins, and other hormones such as those stimulating wound growth, or the postulated hormones of flowering, etc.

II. Assay Methods

Like vitamin assays, auxin assays can only be reliably carried out with auxin-deficient test objects. The most convenient of these is the dark-grown oat coleoptile from which the tip has been removed.

A. Avena Test

As developed by Went and modified by numerous workers this is carried out as follows:

1. Seeds of a pure line (the variety “Victory” or “Segerhavre” is the most commonly used) are husked, soaked for two hours in water, and laid out on wet filter paper with the embryo upward for 24 hours at 25°C. in weak red light.

2. When the roots are about 2 mm. long they are planted in glass holders (see Fig. 1) with the root dipping into water contained in a zinc or glass trough. The holders are held in brass clips in rows of twelve. They can be adjusted in two planes so that the shoots can be made strictly vertical. Some laboratories prefer to grow the plants in sand or soil, either in individual vials or in long narrow boxes.

3. The seedlings are allowed to grow for about 48 hours at 25°C in a dark room. The humidity must be controlled at 85–90% (relative) both to avoid drying and shrinkage of the agar blocks, and because plants grown in lower humidities are less sensitive (Gorter and Funke, 104), while at higher humidities guttation may occur. Small cabinets have been designed to take the place of a controlled dark room (Avery et al., 18) but the latter is more convenient.

¹ Malic and other organic acids promote growth of the coleoptile at $M/1000$ and below (335a) but only in presence of auxin.
(4) Straight seedlings of the same height are selected and the tips of the coleoptiles, to a length of about 1 mm., removed with sharp scissors (stage B in Fig. 2). This and all subsequent operations are carried out in orange or red light free from wavelengths shorter than 5900 Å. Shorter wavelengths, except at extremely low intensities, produce phototropic curvature.

(5) Blocks of agar containing the substance to be tested are made by melting 3% agar and mixing with an equal volume of the test solution. (Formerly blocks of pure washed agar were soaked in the solution but...
this gives unreliable results.) For experiments of the diffusion type, the plant parts are placed directly on 1.5% agar. The blocks are cut up into small blocks of standard size, commonly 10 mm. The size is, however, not critical, since the curvature is essentially proportional rather to the concentration than the amount of the auxin contained in the block (Thimann and Bonner, 318); with 10 mm. blocks, 15% of the amount present enters the plant.

(6) Three hours after decapitation, when growth has slowed down and regeneration of the physiological tip begun (see pp. 12, 32) a further 4 mm. is cut off (stage D, Fig. 2). This is preferably done with special scissors with adjustable closure (see Went and Thimann, 360, Fig. 12). The protruding primary leaf is pulled until it breaks off at the base, but left inside the coleoptile as a support (stage F, Fig. 2).

(7) The agar blocks are placed on one side of the decapitated coleoptile, resting against the leaf (stage G, Fig. 2). From six to twelve or more plants are used for each determination. After a standard time (90 or 110 min.) shadowgraphs of the resulting curvatures (stage H, Fig. 2) are taken. This time is set by the "regeneration of the physiological tip," which causes formation of auxin on both sides and consequent regression of the curvature with increased growth rate.

(8) The curvatures are measured in degrees with a simple goniometer, and from the averages the concentration of auxin in standard units is determined. The plants for each test are calibrated by using blocks containing 0.025 mg. indoleacetic'acid per liter of agar, which gives a curvature within the range of proportionality, and a concentration five times higher, which gives the maximum curvature obtainable. The relation between concentration of auxin and curvature depends on the agar concentrations and the method of preparation of the plants. For times, age, and conditions similar to those given above, this relation is shown in Fig. 3. With higher agar concentrations the proportionality curve does not pass through the origin; with lower concentrations the curve is convex to the abscissa (326). The curvature may also be expressed in terms of $d$, the difference in growth between the two sides. This is done by measuring $r$, the radius of curvature, and $l$, the length of the curved zone, by means of a series of circular arcs drawn on paper. Then:

$$d = \frac{u}{r}$$

where $t$ is the thickness of the coleoptile, usually about 1.5 mm. This method was introduced by Purdy (244) and is used mainly by Boysen-Jensen and co-workers, who also grow their plants in sand or soil rather
than in glass holders. The relationship between $d$ (in mm.) and curvature (in degrees) is approximately linear, a $d$ value of 1 being about $47.8^\circ$.

Although the dark-room conditions are essentially constant, the sensitivity of the test varies with the time of day, being highest in the early morning (167,360). In spite of several attempts (167), no explanation has been found for this. In carrying out the test in diffuse light,

![Graph](image)

**Fig. 3.**—Curvature (dotted) and straight growth (solid line) of *Avena* coleoptiles as a function of the amount of auxin applied. (After Thimann and Bonner, 319.)

Söding and Funke (293b) found the sensitivity lower in warm weather than in cold, although this is not a direct effect of temperature.

**B. OTHER CURVATURE TESTS USING AGAR BLOCKS**

The characteristic feature of the *Avena* test is the use of an agar block of small volume. This makes possible the determination of very small quantities of auxin. In the standard test above, 0.025 mg. indoleacetic acid per liter of agar gives a curvature of about $10^\circ$, measurable to about $10\%$. The amount of indoleacetic acid in each block of volume 10 mm.$^3$ is thus $2.5 \times 10^{-7}$ mg. or 0.0025 γ.

The "deseeded" test (Skoog, 271) uses the oat coleoptile as above, but the seeds, *i.e.*, the endosperms, are removed, without damaging the embryo, at twelve to eighteen hours before the test. The plants are held in the holders by cotton wool. Since the endosperm of the seed furnishes the precursor which is converted to auxin in the regenerated physiological tip (see pp. 22-24), these seedlings do not show regeneration. Hence, the curvatures continue to increase up to six hours after application, and consequently the test, if curvatures are recorded at six hours, is three to five times as sensitive as the ordinary *Avena* test.

The *Cephalaria* test (Söding, 291,293) is carried out in diffuse daylight with decapitated hypocotyls of *Cephalaria*. Because this seedling has a
solid structure, unlike the hollow coleoptile of the grasses, it is less easy to apply the agar to one side. Accordingly the hypocotyl is cut through obliquely and the block placed at the lower end of the cut. The sensitivity of the test has an unaccountably large variation with the season: in June and July it is 400 times as sensitive as the \textit{Avena} test, but in winter it is only about half as sensitive, according to Söding (293). It has been little used.

A curvature test with \textit{Raphanus} hypocotyls was worked out by van Overbeek (226). The two cotyledons were removed, and in their place agar blocks were applied to the petioles—plain agar to one and the test block to the other. The curvatures were photographed after two hours.

\section*{C. Straight Growth Measurements}

Since the action of auxins in nature is to control straight growth, it is in principle desirable that assays should be checked by straight growth measurements, if not actually founded upon them.

Straight growth of rapidly growing organs is readily measured over short periods with a travelling microscope. In this way Söding (288) demonstrated "regeneration," \textit{i.e.}, renewed auxin formation in the coleoptile stump some hours after decapitation. At Utrecht an automatically recording growth-measuring device, or "auxanometer," has been used in some critical studies (\textit{e.g.}, that of Dolk, 78). Measurements of enlarged photographs taken at intervals during growth were used by Thimann and Bonner (319) and showed, \textit{inter alia}, that straight growth, like curvature, increases with the applied auxin concentration up to a clearly-defined maximum (see Fig. 3). Straight growth of decapitated coleoptiles has been used for comparing the activity of different auxins (260). Decapitated \textit{Lupinus} seedlings almost stop growth when exposed to light, and if auxin is applied to them, the resultant elongation, for whole hypocotyls, is linearly proportional to the logarithm of the concentration (108), while short sections show a direct linear relationship very much like that of Fig. 3 (Dijkman, 76).

Straight growth of isolated coleoptile sections is conveniently measured by placing the sections on fine glass rods (Bonner, 30; Thimann, 310) or better still on the teeth of fine combs (Schneider, 262) and floating these on the test solution (see Fig. 4). Sections of coleoptiles growing vertically on agar have been used by Monselise (209) and this method can be used with the auxin in agar blocks like the tests in B and C above. Decapitated, isolated coleoptile sections, growing vertically, have also been used by Funke (88a) for assay of the growth inhibitor of maize seeds.

When the sections are used in solution the pH must be brought to 6.0, because acid pH increases the growth by increasing the fraction of
the auxin in undissociated (as opposed to salt) form (31,259a). The sections should not be submerged in the solution, but should break the surface (335a).

![Fig. 4.—Sections 3 mm. long cut from coleoptiles, mounted on combs and immersed in solutions in petri dishes, photographed after 90 hours. Left: sections in water; elongation about 10%. Right: Sections in auxin, sucrose, and KCl; elongation about 100% with some growth in thickness. (From Schneider, 262.)](image)

**D. CURVATURE OF SLIT ORGANS**

It was found by Went (351) that the internodes of pea stems, if slit lengthwise and immersed in auxin solutions, curve inward (toward one another), the curvature being more nearly proportional to the logarithm of the auxin concentration than to the concentration itself. Jost and Reiss (150) used slit dandelion flowerstalks; and Thimann and Schneider (328) found slit coleoptiles of oats or corn very sensitive. *Helianthus* hypocotyls have been used by several workers, especially Diehl *et al.* (75), but with the auxin applied in lanolin paste. With all such objects, in water alone the halves curve outward, due to tissue tension. In very dilute auxin solutions there is often a slightly increased outward curvature, more marked with some auxins than with others (327). Acid pH has the same effect, probably due to liberation of auxin at the cut surface (31). The inward curvature is, like the curvature of *Avena* coleoptiles in the test under A above, due to a difference in growth between the two sides of the organ, the outer side growing more than the inner (van Overbeek and Went, 238), but in this case the auxin is applied symmetrically and the differential response is inherent in the plant tissue. Van Overbeek and Went concluded that the curvature is due to differ-
ences in the rates of auxin entry on the two sides, entry taking place more readily through the outer intact side than through the central (wounded) tissue, but this has been disproved by Jost (149) and Thimann and Schneider (327), and the exact cause of the differential response has been the subject of considerable study. It is clear that it involves: (a) a true difference in the ability of the different layers of tissue to grow, the epidermis and outer cortex growing more, in response to auxin, than the pith and central layers (75, 149, 327); (b) a retarding effect on growth induced by the longitudinal wounding (263, 353). The response of the epidermis is particularly important, "peeled" plants giving consistently smaller curvatures. The different response to auxin of the different layers is the main cause of the curvatures, and is also responsible for the development of the tissue tensions in the normal growing stem. The method is convenient where sufficient quantity of solution is available, and has been used in chemical studies on the activity of synthetic auxins (see Section III, C). It can be carried out in diffuse daylight.

A modification in which coleoptiles are slit into quarters instead of halves (328) gives considerably greater sensitivity. According to van Santen (259a) this method is much more sensitive to auxin $a$ than to indoleacetic acid, but this is open to question.

E. Epinastic Curvature of Petioles

In many dicotyledonous plants, the angle between the stem and the petiole is constant and characteristic, provided the plant is vertical. Application of auxin dissolved in lanolin to the upper side of the petiole will cause it to be depressed and the increased angle between stem and petiole can thus serve as basis for an assay method. Hitchcock (136) and Hitchcock and Zimmerman (138) have used this method with tobacco and other plants. (The curvature of the petiole in nature is classified as an epinasty and not a geotropism because, although caused by gravity, it is not a curvature toward or away from the earth, but toward or away from the stem. The direction of curvature is thus determined by the structure of the organs concerned.) It is to be presumed that the curvature is due to acceleration of growth on the side to which auxin is applied as in the other tests above, though analysis of the curvature in this sense has not been made. It is well to point out that tests such as this with intact green plants growing in the light are open to an important objection, namely that the test object is already rich in auxin, so that applied substances, even if they have no true activity, may give an effect through an action on the auxin already present. It is probably for this reason that some organic acids, which are not auxins at all, show activity in this method. Relative activities
of different auxins in causing epinasty are roughly in the same order as for causing curvature and gall formation in green plants, but not the same as for the *Avena* test (122).

**F. Other Methods**

Methods depending on the formation or inhibition of roots or buds will be discussed in appropriate sections below. A few of these have been used as assay methods in the past but at present they are used mainly in the studies of the phenomena concerned and not as assays. *Avena* coleoptiles have occasionally been used, intact or decapitated, with the auxin applied in lanolin; the sensitivity is 10–50 times less than with agar (Brecht, 50, Avery *et al.*, 14). In many cases it is desired to assay for a particular type of activity such as growth inhibition of shoots (Section VII, D) or parthenocarpic fruit formation (Section VIII, B). Certain auxins, particularly the alkyl esters of the acids, are effective in the vapor form (375); their action has been assayed by epinasty (above) or by morphogenetic effect on developing buds (see Section III of Chapter III). The swellings produced by applying auxin in lanolin to the decapitated stems of *Vicia faba* seedlings have been utilized for an assay method by Laibach and Fischnich (182). The increase in diameter, measured after four days in the dark, is proportional to the logarithm of the auxin concentration up to a limiting value.

**III. Chemistry of Auxins**

**A. "Auxin A and B"**

In view of the importance of the coleoptiles of the grasses, especially oats, in the early work, it would be expected that efforts would be made to isolate auxins from this material. However, the quantities present are far too small. First steps toward isolation were made by the discovery of auxin in various commercial enzyme preparations by Seubert (269), in cultures of several fungi including *Rhizopus suinus* by Nielsen (216,217), and in human urine by Kögl and Haagen Smit (162 cf. 164).

From the ether-soluble fraction of acidified urine, by an extensive series of fractionations, involving a concentration of 21,000 times, Kögl, Haagen Smit, and Erxleben (163) isolated an acid, termed "auxin a," C_{18}H_{32}O_{6}, and also its lactone. Turning their attention to plant material they analyzed a number of samples of cereal seeds and selected a corn germ oil and a malt sample which appeared to have very high auxin contents. From these were isolated both the auxin a above and a new acid, C_{18}H_{30}O_{4}, named "auxin b" (160). The degree of concentration required was 100,000 times for the malt and 300,000 times for the corn...
oil. The two substances are closely related, the former being a trihydroxy and the latter a ketohydroxy acid:

\[(\text{C}_{13}\text{H}_{23})\text{CHOHCH}_2(\text{CHOH})_2\text{COOH} \quad \text{Auxentriolic acid, "auxin a"}\]
\[(\text{C}_{13}\text{H}_{28})\text{CHOHCH}_2\text{COCH}_2\text{COOH} \quad \text{Auxenolonic acid, "auxin b"}\]

The "auxin a" lactone is considered to have the 1,5-lactone form.

In spite of the small amounts available (less than 1 g. of total active crystals), Kögl and Erxleben established the structure of the C$_{13}$ residue as 2,4-di-sec-butyl-Δ$^1$-cyclopentene and confirmed this by the identity of the substituted glutaric acid, obtained by oxidative breakdown, with a synthetic product, 2,3-diisobutylglutaric acid, II. The full formula of auxin a is therefore I:

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 \\
\text{C}_8\text{H}_6\text{CHCH—CCHOHCH}_2\text{CHOHCHOHCOOH} & \quad \text{H}_2\text{C} \\
\text{CH}_3 & \quad \text{CH}_2 \\
\text{C}_8\text{H}_6\text{CHCH—CH} & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{CH}_2 \\
\text{C}_8\text{H}_6\text{CH—CH—COOH} & \quad \text{II}
\end{align*}
\]

On standing in the dark, the double bond shifts to the side chain and the hydroxyl to the ring to produce an inactive substance, pseudoauxin a (p. 19). Auxin a lactone undergoes a similar change with loss of water. This and related changes may play a role in phototropism (see Section V).

B. INDOLE-3-ACETIC ACID

In extending their work on urine, Kögl, Haagen Smit, and Erxleben (165) found that a large part of the auxin present was destroyed by attempts to lactonize with hydrochloric acid in methanol. A modified isolation method thereupon led to the identification of indole-3-acetic acid as a third active compound. Its activity in the Avena test is probably about half that of auxin a or b. Kögl and Kostermans (168) also isolated this substance from yeast plasmolyzate.

Working independently on the auxin produced by Rhizopus suinus cultures, Thimann (310) showed by isolation that this also is indole-3-acetic acid. At first it was thought that indoleacetic acid is typically a
product of microorganisms and not a true hormone of higher plants, and it was accordingly named by Kögl et al. "heteroauxin," but Haagen Smit and co-workers (125) subsequently isolated it in pure form from alkali-hydrolyzed corn meal and indicated that most of the activity of the hydrolyzed meal was due to indoleacetic acid rather than auxin a. Haagen Smit et al., (124) later obtained it also from the endosperm of immature corn grains. Gordon and Wildman (102,103) have brought forward evidence that alkali treatment produces traces of indoleacetic acid from the tryptophane in a number of proteins (see below), but this is not likely to be the main source of the indoleacetic acid isolated. Instability to hot acid and stability to alkali indicate that the auxin extracted from many higher plants (237, and see Section D below) is of the indole type. It is probable, therefore, that indoleacetic is widely distributed in higher plants, perhaps more widely than auxin a and b. and it is evidently a true plant hormone. The high specificity of the indoleacetic-acid-inactivating enzyme of the pea plant (306) also points in this direction; some workers believe auxin a and b occur only rarely.

Besides indoleacetic acid, indoleacetaldehyde also occurs in plants, particularly in dark-grown Pisum, Vicia, Helianthus, and Brassica (187). The aldehyde is readily oxidized to the acid by Schardinger's enzyme from milk, or by contact with soil. It behaves as a "neutral auxin," and was discovered through its presence in the neutral fraction by Larsen, who purified extracts by shaking out from ether at different pH. Its identity was established by conversion to indoleacetic acid and various other tests. Its widespread occurrence is, of course, further evidence for the importance of indole derivatives as plant hormones.

C. SYNTHETIC SUBSTANCES NOT KNOWN TO OCCUR NATURALLY

A great number of related compounds have been prepared and tested. The results depend to some extent on the assay used. The Avena test is highly specific. Besides the above compounds, only the lower alkyl esters and two of the methyl derivatives of indoleacetic acid (169), the isostere indene-3-acetic acid (311), and indole-3-butyric and 1-naphthaleneacetic acids (360), show appreciable activity in this test. The potassium or sodium salts show about the same activity as the free acids, provided the solutions are not buffered. A few other substances show activity in very high concentrations only, frequently producing very short apical curved zones. Phenylbutyric acid, which is inactive by itself, inhibits the effect of indoleacetic acid, by competition (272a), or perhaps by a more complex mechanism. This substance (and also cyclohexaneacetic acid) greatly increases the auxin curvature in the pea test (353). This is explained by Went (353,356) in terms of two proces-
ses; one, the “preparatory” reaction, can be carried out by substances inactive as auxins, while the other, the “growth” reaction proper, requires the chemical structures discussed below.

Straight growth of isolated stem or coleoptile sections (see Section II, C) is less specific, and the curvature of immersed slit stems (Section II, D) or other methods still less so. Hence the activity of a given synthetic auxin, relative to a standard such as indoleacetic acid, varies with the type of test. This is illustrated by Table I (from Thimann and Schneider, 1939), which not only shows the difference in specificity of the tests, but also illustrates how compounds inactive, or almost so, in one test may show high activity in others. However, the order in which the substances fall is nearly the same in each test (see also the data of Gustafson, 122).

Using the slit pea stem curvatures, Haagen Smit and Went (126) and Koepfli, Thimann, and Went (157) have tabulated the activities of a large number of related compounds, and Veldstra (1944a) has added a number more. Using epinasty and the changes in shape of young tomato leaves, Zimmerman and Hitchcock (372) and Zimmerman (370) have added a further large group, including the highly active ring-

<table>
<thead>
<tr>
<th>Acid</th>
<th>Curvature of slit stems of <em>Pisum</em></th>
<th>Straight growth of <em>Pisum</em> sections</th>
<th>Straight growth of <em>Avena</em> sections</th>
<th>Curvature in standard <em>Avena</em> test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene-1-acetic</td>
<td>370</td>
<td>23</td>
<td>15</td>
<td>2.5</td>
</tr>
<tr>
<td>Indole-3-butyric</td>
<td>190</td>
<td>22</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Indole-3-propionic</td>
<td>150</td>
<td>8</td>
<td>1.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Phenylacetic</td>
<td>10</td>
<td>0.4</td>
<td>0.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Benzofurane-3-acetic</td>
<td>6</td>
<td>0.3</td>
<td>0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Phenylbutyric</td>
<td>3</td>
<td>0.08</td>
<td>0.06</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* Activity of indole-3-acetic acid brought to 100% for each test. (From Thimann and Schneider, 328.)

substituted derivatives *p*-chloro- and 2,4-dichlorophenoxyacetic acids. Some approximate relative activities for the induction of seedless fruit (see Section VIII) have been given for these compounds by Zimmerman and Hitchcock (373). As might be expected, the ratios of the activities of various substances determined in this way are not the same as by the above methods.

In spite of all this work, it is still not possible to make a really binding
statement as to the structural requirements for auxin activity. The difference between the tests, mentioned above, is in part due to the necessity for the substance to be transported through the plant tissue in tests using agar blocks, but not in tests using immersion in a solution. Some substances, though highly active locally, are not readily transportable. This important limitation, brought to light with indeneacetic and benzo-furanaeacetic acids by Thimann (311), was confirmed for several substances by Went and White (361) in transport experiments, which are discussed in Section IV, A. Then, too, the stability of the substance to plant enzymes, its permeability through cell membranes, and the fraction present in undissociated form (29,31) all influence the responses, the last because the ionized salt form does not penetrate into the cell readily, as shown by Albaum et al. (4). A correction for the extent of dissociation increases the apparent activity of many substances in the pea test. The influences of these modifying factors are discussed in Went and Thimann, Chapter 8 (360), and by Went (353,355), and more recently by Veldstra (339). The auxin-inactivating enzyme in pea plants is highly specific for indoleacetic acid (Tang and Bonner, 306); this might cause this substance to show a lower activity than other, unnatural, compounds.

These factors can as a first approximation all be considered secondary, the primary one being the ability to cause cell enlargement when present in the cell. Using this criterion of primary activity, Koepfl, Thimann, and Went (157) stated the following structural requirements: (1) A ring system as nucleus; (2) A double bond in the ring; (3) A side chain containing a carboxyl group (or an ester or amide readily convertible to a carboxyl); (4) A distance of at least one carbon atom between this group and the ring; and (5) A particular space relationship between the carboxyl and the ring.

As to 1, no aliphatic compounds tested have shown activity.

As to 2, dihydroindoleacetic acid and dihydroauxin are inactive; so is cyclohexane acetic acid. A number of compounds with unsaturation in the side chain but not in the ring, such as pseudoauxin, III, cyclohexylideneacetic acid, IV, and benzofulveneacetic acid, V, are inactive.

As to (3), some modification is needed to allow for the small but definite activity of naphthyl-1-nitromethane (aci form), VII, and indican,

\[
\begin{align*}
\text{III} & : \quad \text{C}_4\text{H}_4\text{CH} - \text{C} = \text{CHCH}_2(\text{CHOH})_2\text{COOH} \\
\text{CH}_2 & \\
\text{C}_4\text{H}_6\text{CH} - \text{CHOH} & \\
\text{IV} & : \quad \text{CH}_2 \quad \text{H}_3\text{C} \quad \text{CH}_2 \quad \text{H}_3\text{C} \quad \text{CH}_2 \\
\text{V} & : \quad \text{CH}_2 \quad \text{H}_3\text{C} \quad \text{CH}_2 \quad \text{H}_3\text{C} \quad \text{CH}_2 
\end{align*}
\]
VIII, both of which have acid side chains which are not carboxyl groups (339). It may be that any acidic (i.e., hydrogen-ion-yielding) group is effective to some extent. Also napthaleneacetonitrile and tryptamine (271) show a slow activity, which is doubtless due to conversion to a carboxylic group within the plant. There is some evidence, however, that naphthalene-1-acetamide, IX, is active without being hydrolyzed (335a).

The activity of esters is not entirely clear. The data of Kögl and Kostermans (169), with the Avena test, show decreasing activity with increasing molecular weight of the alkyl-esterifying group of indoleacetic acid; they therefore concluded that activity was due to hydrolysis (by plant esterases) to the free acid, which should go with decreasing rapidity as molecular weight of the alkyl group increases. Avery et al. (14) have found the esters to have about the same activity as the free acids, or somewhat less in the case of naphthaleneacetic acid; this would agree with the above view. However, Zimmerman and Hitchcock (371,374) found, in experiments with tomato plants, that at least the methyl ester of indolebutyric acid has slightly higher activity than the free acid. This might, of course, be due to some secondary property of the ester such as ease of penetration through the intact epidermis. By contrast, the esters of auxin a are inactive in the Avena test (160).

As to 4, the optimum distance is commonly one carbon atom, activity decreasing with increasing length of side chain, but there is some alternating effect, indolebutyric being more active than indolepropionic acid. The carbon atom may be replaced by other hetero atoms. In the case of phenoxy and napthoxy acids the hetero atom oxygen is present as well as the one carbon atom.
Point δ is the most ill defined. The activity of cis-cinnamic acid and some of its derivatives, and the inactivity of the trans isomers, are among the main pieces of evidence. In the Avena test, the two optical isomers of α-(β-indole)-propionic acid, X, have different activity, the (+) being thirty times as active as the (—) (171), but, since the activity on immersed coleoptile sections is identical, this difference apparently does not relate to primary growth activity. It provides another example of the high specificity of the Avena test. Veldstra (339) has postulated that the side chain must be perpendicular to the plane of the ring, and supports the argument by consideration of molecular models. He makes clear that in cis-cinnamic acid the side chain is perpendicular to the plane of the ring, while in the trans form both are in the same plane. Even in napthalene-1-acetic acid the position perpendicular to the ring is favored. Yet it is difficult to see how introduction of halogen atoms into the ring could alter such spatial relationships. Thus Zimmerman showed, with epinasty (370), that introduction of the ortho chlorine atom increased the activity of phenoxyacetic acid twenty times, the para chlorine atom by eighty times, while both together (2,4-dichloro derivative) increase it some 1200 times. In the pea test (335a) these four substances have the following activities, as per cent of that of indoleacetic acid:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenoxyacetic acid</td>
<td>ca. 0</td>
</tr>
<tr>
<td>α-Chlorophenoxyacetic acid</td>
<td>4</td>
</tr>
<tr>
<td>p-Chlorophenoxyacetic acid</td>
<td>200</td>
</tr>
<tr>
<td>2,4-Dichlorophenoxyacetic acid</td>
<td>1200</td>
</tr>
</tbody>
</table>

While substitution in the ortho position might possibly have some effect in orientation of the side chain, it seems hardly likely that substitution in the para position could do so. There are numerous other examples of the same effect. The exact nature of the spatial relationships must therefore be left open for the present (but see Wisconsin Symposium, 1951).

D. NATURE OF AUXIN PRECURSORS

The auxin in human urine clearly comes from the diet. The esters of auxin α are inactive, and some oils yield auxin on hydrolysis with
lipase or with sodium ethylate (160). Ingestion of natural oils increased the auxin content of urine, while hydrogenated oils, pure protein, and sugar did not (163). Indoleacetic acid in urine, similarly, comes from ingested protein (125), wheat giving a particularly clear rise in urine auxin as soon as one hour after feeding.

As was mentioned in Section II, the auxin produced in the coleoptile tip, or in the apical part of the stump in "regeneration," is formed from a precursor in the seed. This was first made probable by Cholodny (61), who showed that soon after the seed was wetted auxin appeared. This auxin does not, as claimed by Pohl (242), travel directly up into the coleoptile, but that which goes into the coleoptile tip travels up as an inactive precursor. This was shown by Skoog (271), who placed agar blocks for a while on the stump of decapitated coleoptiles and then showed that when applied one-sidedly to freshly decapitated coleoptiles ("deseeded test") they caused no effect at first but slowly induced a curvature after two to six hours (341).

Following the work of Thimann and Skoog (332), Gustafson (115,118), Wildman and Gordon (365), and Thimann, Skoog, and Byer (333) on the extraction of auxin from plant tissues, it has now become increasingly clear that many plant materials yield auxin very slowly on extraction with ether, and that this auxin stems from proteins in the tissue. The slow yield is due to a reaction with water, probably proteolysis, which liberates auxin. This reaction is stopped by boiling (332) and this has been put to use for an assay of the free auxin in plant tissues by Gustafson (118). It is also stopped by thorough drying (197,332) and at once resumed on adding water. Proteolytic enzymes, especially chymotrypsin, were found by Skoog and Thimann (274) to accelerate greatly the liberation of the auxin. Wildman and Gordon (365) and Gordon and Wildman (103) have obtained an auxin which is almost certainly indoleacetic acid from isolated plant proteins both from leaves and from seeds. Since this auxin is best obtained by alkaline hydrolysis (25), some of it, at least, doubtless derives from oxidative deamination of tryptophan. However, this is probably not the whole story, for two good reasons: (a) in the case of cabbage leaves the auxin yields are probably too high to be ascribed to the tryptophan present, according to the determinations of Avery, Berger, and White (15); and (b) auxins which may be either acid labile (i.e., indole derivatives) or alkali labile (presumably auxin a or b) may be obtained from purified wheat proteins (Gordon, 102). It is to be noted that Gordon's wheat proteins were well characterized, which makes it highly improbable that the auxin could be merely an impurity. In any event, particularly in the case of auxins liberated by enzymes, there is no reason to doubt that, as was originally
postulated (333), true auxin-protein complexes do occur. These could, of course, serve as important auxin reserves for the plant.

The form in which auxin occurs in seeds differs from that in other tissues. The bulk of the auxin in the cereal grains is in bound form, in the endosperm, and only liberated by alkaline hydrolysis (13,125,130, 333). It is this material which is indoleacetic acid, as shown by Haagen Smit et al. (124,125) and Berger and Avery (24,25). The quantities are large enough in corn, i.e. 20-100 mg./kg., that it acts as an antivitamin in animal growth (156). However, some auxin is obtainable, largely from the embryo, by direct extraction with organic solvents, as in the isolations by Kög and co-workers described above, and this material is auxin \( a \) and \( b \). Thirdly, the addition of water to the endosperm liberates a moderate quantity, presumably by enzymic action. Much of this was probably also bound in the dry state, either chemically as a precursor, or in some physical or adsorptive manner, as in dried Lemna, in which the auxin can be first liberated and then made unextractable by drying (332). Whether the water-extractable auxin in the grain is auxin \( a \) or indoleacetic acid is not clear. Hatcher (130) has assumed that it represents free auxin, the alkali-hydrolyzable part being the bound or precursor form, but there is not enough evidence for this yet. The situation is complicated by Funke's finding (88a) that part of the auxin in corn endosperm is stable to hydrogen peroxide.

In contrast to the grains, no auxin is liberated from Lemna by alkali autoclaving, although, as with other green tissues, it is set free slowly by moist ether (118,332), as discussed above. Cabbage (15) and spinach, however, do yield some auxin to alkali, though in the author's unpublished experiments spinach leaf proteins gave much higher yields with chymotrypsin than with alkali. The purified auxin-protein in spinach leaf cytoplasm does not liberate its auxin readily; it is resistant even to vigorous electrodialysis, and sets free auxin only when actual proteolysis occurs, so that it is indeed a relatively stable complex (Bonner and Wildman, 35). Between these two extremes there seem to be many intermediate states, in different tissues, in regard to ease of liberation (309,333).

A true precursor, of course, would be a substance from which auxin is continually produced, by plant enzymes, under normal conditions and in physiologically significant amounts. It is by no means certain that the auxin-proteins fulfil these criteria. Neither papain-hydrogen cyanide nor the enzymes of autolyzing Lemna liberated any appreciable amount of auxin (333), and chymotrypsin, as far as is known, does not occur in plants. Ficin, which does liberate auxin from Lemna, is a plant enzyme, it is true, but it is not known to be widely distributed. The partial
liberation of auxin on slow drying of leaves may be enzymic, but it is quantitatively rather small. A true precursor system was, however, studied by van Overbeek (233) in the isolated coleoptile tip, which continues for a long period to yield auxin to agar blocks, although the amount which can be extracted from it by organic solvents at any time is relatively small (309). Berger and Avery (23,24) made a partial isolation of a true auxin precursor from corn; this appears not to be a protein, having only 4.7 to 6.4% nitrogen, but its nature remains unknown. It yields indoleacetic acid on alkaline hydrolysis. The variation in amount of precursor and "free auxin" (but see comment above) with age and drying of the grain in rye has been very thoroughly studied by Hatcher (130), who finds that the "free" form appears first and then decreases as the bound form increases and the grain ripens. Some of these changes may, however, be due to variations in the amounts of inhibiting substances rather than in the true auxin (88a).

A more remarkable precursor was obtained earlier from radishes by Stewart, Bergren, and Redemann (297,299); this substance in the intact form actually inhibits growth of the Avena coleoptile, giving marked positive curvatures, but on hydrolysis yields an auxin which is probably indoleacetic acid. Its chemical nature is also unknown but it is thought to be a peptide. The further study of this substance might be important in regard to inhibitions (see Section VII).

The ability to convert tryptophan to indoleacetic acid is probably widespread among microorganisms; this is doubtless the source of the auxin in fungus cultures, as shown by Thimann (310). Furthermore, this is the most probable source of the large amounts of auxin produced in bacterial infections of plants such as legume root nodules and crown galls (see Section VIII, A). Other plant infections resulting in pathological overgrowth (188,189) may have the same explanation, and indeed Link et al. (197) have shown that aphids are very rich in auxin; whether this was extracted from the leaves on which they fed, or elaborated within the aphid is not clear, but in any event either the removal or injection of auxin by the aphid may account for some of the growth effects caused by these parasites. As to higher plants, the evidence as to their ability to convert tryptophan to indoleacetic acid under natural conditions is not perfect. Tryptophan causes a slow curvature in the "deseeded" Avena test; it causes straight growth of coleoptiles when applied to the base but not to the tip, and it leads to root formation on pea cuttings (335,335a). On the other hand it cannot replace indoleacetic acid in sterile tissue cultures, as found by Nobécourt (221). Unless the tests with higher plants are carried out under sterile conditions a positive result might always be due to infection. Because the growth effects produced by tryptophan
differ anatomically from those caused by indoleacetic acid. Kraus (174) claims that its action cannot be due to conversion to the latter compound. But since only one concentration, in lanolin, was studied in his experiments, and since growth effects are characteristically dependent on concentration, this conclusion is obviously unjustified. The best evidence of conversion is that of Wildman et al. (364a), who obtained formation of an active auxin by spinach leaves infiltrated with tryptophan within two to four hours. The enzyme system was present in dialyzed cytoplasm prepared from the leaves and had its optimum pH at 7.5. There is some evidence that the reaction goes via indolepyruvic acid (cf. 310); in any event it is an oxidative process.

The case of tryptamine, which, like the precursor in the seed, is directly converted to auxin in the Avena coleoptile (271), is worth special mention, though its biological significance is not known. Lastly the indoleacetaldehyde found by Larsen (187) in etiolated Pisum and other plants must be considered under this head. Larsen's extracts of neutral auxin, which had quite low activity, were converted to highly active material, considered from diffusion measurements to be indoleacetic acid, by treatment with soil or with a preparation of Schardinger oxidase. In some cases the neutral material had no growth activity at all, which suggests that there is more than one neutral compound convertible to indoleacetic acid. There is no evidence here, though, that enzymes in the plant can carry out the conversion. Hemberg (131a) finds a similar situation in potato tubers.

An interesting general scheme for auxin activity has been proposed by Skoog, Schneider, and Malan (272a), according to which the auxin molecule, envisaged as a kind of coenzyme, has to combine on the one hand with its substrate and on the other with an apoenzyme. Precursors could thus be of two kinds: those in which the substrate-combining part is covered or distorted but can be corrected by the plant, e.g., tryptamine or indoleacetaldehyde, and those in which the apoenzyme-combining part has been combined with some other molecule but can be freed under some conditions. The latter have their substrate-combining activity intact and can therefore occupy the substrate to the exclusion of free auxin molecules, thus giving competitive inhibition (e.g., phenylbutyric acid) or even total inhibition (e.g. the inhibitor of Stewart et al. described on p. 24). These authors point out that, if excess auxin were present, some molecules would combine only with the substrate and some only with the apoenzyme “thus effectively blocking each other from reacting.” This would account for inhibitions of the type discussed in Section VII, A. This ingenious scheme has much to recommend it, though considerably more evidence would be needed to establish its validity.
IV. Transport of Auxin

A. Polar Transport and Its Mechanism

One of the most remarkable properties of living plant tissue is the strictly polar way in which auxin is transported in it. The polarity of shoots, particularly in regard to bud development and root formation, has been recognized from very early times, and the polar transport of auxin provides an explanation for at least many such phenomena. The earlier work on polar transport of auxin has been so fully reviewed (360, Chap. 6) that it needs only the briefest recapitulation here.

In seedlings, phototropism is detected by the tip and the stimulus conducted toward the base; movement in the reverse direction does not occur. Interposition between the tip and base of a section of inverted tissue prevents the movement (see Fig. 5, II), which is therefore strictly basipetal. Auxin will be transported directly through a short section of Avena coleoptile in the apex-to-base direction, but not inversely (Fig. 5, I). The process is not one of diffusion, as was proved by the experiments of van der Weij (346), which were carried out as shown in Fig. 5, I, the auxin in the blocks being determined by the Avena test. The main results can be summarized as follows:

---

**Fig. 5.**—I. Diagram of transport experiment. Auxin is transported from agar block A through coleoptile section B to receiving block C. Left, normal transport; right, section inverted, no transport. Degree of shading indicates auxin concentration in agar.

II. Transmission of phototropic stimulus through normal (left) and inverted (right) section of coleoptile introduced between tip and base of another coleoptile. (From Went and Thimann, 360.)
The temperature coefficient of the amount transported per unit time between 0° and 30°C is about 3, i.e., that of a chemical reaction. The velocity, however, as measured by the time taken for the first auxin to appear at the basal end of the conducting tissue, is about 12 mm./hour in *Avena* and is independent of temperature. This is determined by extrapolation (see Fig. 6). The concentration of auxin in the agar block at the receiving end soon equals that in the donating block, and subsequently exceeds it, so that auxin must be actively transferred against its gradient. By etherizing the sections, polarity disappears and with it also disappears the "active" nature of the transport; it now becomes essentially a diffusion process.

Auxin transport is thus like that of "objects along a moving band; the band goes at constant speed so that the number of objects arriving at the end per unit time is independent of the length; the time required for the first object to reach the end is proportional to the length of the band; if not removed from the end the objects continue to pile up" (Went and Thimann, 360). Stems (see Beal, 21), petioles, hypocotyls, and leaf veins behave like coleoptiles so far as they have been studied. Tissue cultures, especially of carrot and endive, demonstrate the polarity of auxin transport in many ways (Gautheret, 98, pp. 161-166). Other auxins than indoleacetic acid move both more slowly and in smaller quantities per unit time. The data of Went and White (361) yield the following rates in millimeters per hour through *Avena* coleoptiles:

4 Unpublished experiments of W. P. Jacobs show that the polarity is far from strict in young *Phaseolus* hypocotyls.
Indoleacetic acid ........................................... 9.0
Indolebutyric acid ........................................ 6.6
Anthraceneacetic acid .................................... 5.4
Napthaleneacetic acid ..................................... 3.9
cis-Cinnamic acid .......................................... Not detectable

It should be added that longitudinal transport of auxin is not affected by light (226); this is important for the understanding of phototropism (see Section V).

The mechanism by which this active transport is achieved is not understood. Accumulation of solutes against a gradient, as by roots or by algae growing in very dilute nutrient solutions, must involve a comparable type of active transport (352), but in this case in the lateral rather than the longitudinal direction. Arisz has recently brought to light (10) a similar transport of amino acids through the tentacles of Drosera, and Schumacher (265) described polar movement of fluorescein in stem hairs of cucurbitis. The polarity of auxin transport is therefore not an entirely isolated phenomenon.

Attempts have been made to relate the transport to the electrical polarity of the plant. The apex of shoots is in general negative to the base, as shown by the early work of Lund (see 200) with nonpolarizable electrodes. This apical negativity is still present in short sections of stems or coleoptiles, and is largely abolished by etherization (64). The anion of a weak acid such as auxin would, of course, be transported from apex to base under such a potential. Koch (153) showed that plant auxin in agar does in fact move toward the anode, and Clark (63) confirmed this for pure indoleacetic acid. Kögl et al. (167) showed essentially the same thing by making the agar block in the Avena test negative to the plant, and passing a small current, which had the effect of increasing the resulting curvature, doubtless by increasing the movement of auxin from the agar into the plant. Then, too, coleoptiles and shoots placed in air or water between oppositely charged poles curve toward the positive pole (6,49,153); such curvature implies more growth on the side toward the negative pole. Electrolytic movement of auxin has even been produced directly in plant tissue by Koch (153), by inserting electrodes into opposite sides of sunflower hypocotyls, which were subsequently halved and tested for auxin (by applying them to roots). The hypocotyls here curved toward the negative pole and the convex half gave the greater curvature on the test roots. These experiments all show that electrolytic movement is possible, and takes place in the right direction. But here the parallel ends, for the following reasons: (1) a potential gradient of 50 volts/cm. was needed for detectable transport—far higher than the electrical gradients observed in plants, (2) externally
applied potentials do not affect the polarity of auxin transport through coleoptile sections, even though they may reverse the electric polarity, (5) inverting the section with respect to gravity inverts the electrical gradient but does not affect the auxin transport (63), and (4) treatment with 10–100 p.p.m. of sodium glycocholate completely abolishes the transport but does not affect the electrical polarity, or indeed any other observable property of the coleoptile section (see Table II; from Clark, 64).

The absence of any effect of low glycocholate concentrations on respiration, while auxin transport is wholly prevented, is of interest since

| TABLE II |
| Effect of Glycocholate on Auxin Transport and Electrical Polarity of Coleoptile Sections |

<table>
<thead>
<tr>
<th>Sections infiltrated with</th>
<th>Units auxin transported in 2 hr.</th>
<th>Emf between apex (−) and base (+), mv.</th>
<th>Protoplasmic streaming</th>
<th>( Q_{O_2} ) (in separate expts.)</th>
<th>Appearance of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>11.4</td>
<td>10</td>
<td>+</td>
<td>1.21</td>
<td>Turgid</td>
</tr>
<tr>
<td>Na glycocholate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 p.p.m.</td>
<td>0</td>
<td>10</td>
<td>+</td>
<td>1.22</td>
<td>Turgid</td>
</tr>
<tr>
<td>100 p.p.m.</td>
<td>0</td>
<td>10</td>
<td>+</td>
<td></td>
<td>Turgid</td>
</tr>
<tr>
<td>1000 p.p.m.</td>
<td>0</td>
<td>0</td>
<td>−</td>
<td></td>
<td>Flaccid</td>
</tr>
</tbody>
</table>

normal respiration is apparently essential for transport of auxin into the section (33). The absence of any inhibiting effect on streaming suggests that transport does not take place in the streaming protoplasm. Similarly, Schumacher (265) could observe protoplasmic cyclosis going on simultaneously with polar movement of fluorescein in the cells of the cucurbit hair.

As will be shown in the following section, curvatures induced by gravity involve a movement of auxin laterally across the coleoptile or stem. Here also it has been thought that an electrical gradient, resulting from gravity, might be responsible, and long ago Brauner (47,48) showed that indeed the under side of a stem placed horizontal becomes electropositive to the upper side (the "geoelectric effect"). The potential difference due to gravity is established before any curvature occurs, and there are several very suggestive relations between the potential and the subsequent auxin transport brought out by Schrank (264). No causal relationship has as yet been established, however.
It can only be concluded that auxin transport is not directly related to electric polarity; it is in some way related to respiratory processes but the link can readily be broken without damaging these processes.

B. Upward Transport

There are two conditions under which auxin is transported upward, *i.e.*, from base to apex. The first is when it is applied to the upward-moving transpiration stream, as by pouring a solution on the soil (137) or adding auxin to a nutrient solution in which stem cuttings (138) or roots (272) are immersed. In such cases, so long as transpiration occurs, the auxin is passively carried upward in the xylem in the same way as salts or dyes and the amount absorbed parallels the absorption of water. It is a function of the transpiration rate but is also influenced by the concentration of salts in solution. Skoog has, however, shown (272) in extensive experiments with tomato stems that auxin taken up in this way then moves laterally into the surrounding living tissues and is re-exported downward by the normal polar transport.

The other condition is when very high concentrations are applied. Went and White (361), taking every precaution to avoid leakage along surfaces, still obtained inverse transport in the coleoptile when concentrations of 1000 mg./l. indoleacetic acid were used. Snow (282, see also 284) obtained curvatures apical to the point of application by using fairly high concentrations in lanolin; the effect was more marked when the application was close to the vascular bundle, so that it probably involved movement in the transpiration stream also. Stewart (298) showed by *Avena* tests that auxin moved upward when very strong (2%) paste was applied to the first internode of a young bean plant. It is probable that these effects are due to the toxicity of high auxin concentrations.

Finally mention may be made of the interesting case of inverted cuttings, *i.e.*, cuttings rooted at the apex, budding from the base, and planted inversely. In such cuttings there is a gradual development of a new series of cells from the shoots to the roots, opposite in polarity to those originally present, and correspondingly Went (357) found that at first the auxin transport is apex-to-base polar, but gradually base-to-apex transport appears as well. Normal cuttings show no such change. This phenomenon only serves to emphasize the strictly polar nature of auxin transport under normal physiological conditions.

V. Role of Auxin in Tropisms

Although it was through tropisms that the role of the “growth substance” was first discovered (see Section I) interest in the past ten years
has shifted away from this aspect. The majority of the facts have been
discussed in detail by Went and Thimann (360); for phototropism the
older literature is treated in extenso by DuBuy and Nuernbergk (56) and
more recent summaries are given by van Overbeek (231) and by Oppen-
orth (225). Only the briefest outline will therefore be given here.

A. GEOTROPISM

Geotropism is the curvature of shoots away from the earth (negative)
or of roots toward it (positive). The latter is not well understood because
the role of auxin in the growth of roots is not clear. The former, how-
ever, is explained satisfactorily by the Cholodny-Went theory, namely,
that when a shoot is horizontal more auxin moves to the lower side than
to the upper; the lower side therefore grows more, causing upward curva-
ture (Cholodny, 60). First worked out by Dolk (78) for coleoptiles, by
allowing the auxin from upper and lower halves to diffuse into two sepa-
rate agar blocks, this experimental analysis of geotropism has since been
generally accepted for all growing shoots; it has been confirmed by several
workers (45,76) and with both extraction and diffusion methods. Inci-
dently it provides one of the best illustrations of the strict limitation of
growth by auxin supply; instead of the two halves each receiving 50% of
the available auxin, the lower receives some 65–70%, and this differ-
ence is sufficient to cause immediate geotropic curvature.

Gravity does not of itself cause any increase in the total growth rate
(“geogrowth” reaction) (78) nor in the auxin production rate (76) or
total auxin content, except in the mature nodes of grasses, which when
placed horizontal begin to form auxin afresh (261); the same phenomenon
occurs in sugar cane (234) and is apparently due to the liberation of free
auxin from a bound form. It is worth noting that “lazy” maize, which
is insensitive to gravity and grows horizontal, does not show the normal
accumulation of auxin on its lower side but accumulates a slight excess
about 55%) on the upper side, as shown by van Overbeek (229) and
Shafer (270); many other prostrate and “lazy” plants, however, show
normal geotropic response (185) (see p. 34). Another interesting
exception is furnished by the action of ethylene, which causes positive
geotropism in shoots of Vicia; here an excess of auxin accumulates on the
upper side instead of the lower (178), so that ethylene must influence the
transverse transport of auxin, a phenomenon extensively studied by
Borgström (36).

It should be added that the auxin transported laterally is only the
free-moving auxin of the coleoptile. This was made clear from Went’s
studies (358) of the relation between diffusible and extractable auxin in

So called because it was proposed by Cholodny and confirmed by Went.
regard to growth and tropisms. After decapitation, the geotropic sensitivity falls to very low values (78) and does not reappear again until new auxin production occurs ("regeneration") 2.5 hours later. The total extractable auxin, however, only falls to about 50% of the initial value before regeneration sets in. On the other hand, the free-moving auxin, determined by diffusion out of the tip, falls, like the geotropic sensitivity, almost to zero, until regeneration starts. Thus it is the diffusible auxin which is redistributed by gravity.

The mechanism by which auxin is transported laterally under the influence of gravity is unknown. Attempts to correlate it with "geo-electric potentials" have been without success, as discussed in Section IV for normal transport. It would seem that gravity can only be perceived by something falling; the older literature ascribed much importance to small starch grains, the "statoliths" of Haberlandt, but as yet no relation between the movement of these and the movement of auxin has been established.

B. PHOTOTROPISM

Phototropic curvatures are more complex, since they vary both quantitatively and qualitatively with light intensity. In the Avena coleoptile, which has been most studied, curvature takes place toward the light (positive phototropism) under low light quantities, away from it (negative) at higher, and toward it again at still higher. For the first positive curvature (at 20–100 meter candle seconds), Went showed in 1928 that more auxin diffuses from the dark side of the tip than from the lighted side. Similarly, for the negative curvature (at 1400 meter candle seconds), Asana found more auxin diffusing from the light side of the tip (11). These results suggest the simple Cholodny-Went theory, namely, that light causes lateral movement of the auxin which is responsible for the curvature. They explain the earlier experiment of Boysen-Jensen (43), who divided the coleoptile tip longitudinally with a fragment of glass; when this was done parallel to the direction of the light, curvature took place, but, when perpendicular to the direction of the light, curvature was prevented, presumably by stopping the lateral transport. Further, the same lateral transport to the dark side was found in seedlings of two dicotyledons: Raphanus by diffusion (226) and Phaseolus by extraction with chloroform (45). Light does not affect the normal longitudinal transport of auxin (226,299a, but cf. 55a).

However, there is another effect, namely, that a given amount of auxin produces more growth in the dark than in the light (226,331). Insofar as low light intensities are concerned, this appears to be due to a destruction of auxin—probably auxin a (166)—by light. In his original
"redistribution" experiments Went (348) found by diffusion less total auxin (dark and light sides combined) after illumination than in dark controls, and this was confirmed with the ether extraction method, both by Stewart and Went (299a) and by Oppenooth (225). The extent of inactivation does not seem to increase very much with time of exposure, at least as far as the data go; one second of sunlight caused about as much inactivation as sixty seconds (299a). The destruction is of the order of 25% and is accompanied or followed by the shifting of the auxin toward the dark side (55a, 225). Longer exposures cause an increased synthesis of auxin (225), which is discussed below.

The mechanism of this effect has been extensively studied by Kögl and colleagues at Utrecht. Koningsberger (173) found in 1936 that auxin a lactone shows ultraviolet absorption due to its very rapid conversion to an inactive product, "pseudo-auxone"; even the weak irradiation needed to determine its ultraviolet absorption spectrum inactivates 80–100% of the auxin activity (161). Since the free acid (auxin a) and its lactone are in equilibrium in weakly acid solution and since only very weak light is necessary, there is here a mechanism for inactivation by light. What is more important is that the inactivation may occur in the visible spectrum through the mediation of suspensions of carotene (170, 266). Both α- and β-carotenes and some other carotenoids are effective. Since carotene is present in the coleoptile (343) and particularly in the apical two millimeters (52), it can hardly be doubted that through this system auxin a is destroyed in situ by light. Further, the spectral sensitivity of the coleoptile to light (19, 148) agrees well with the absorption spectrum of a carotenoid. This is, then, a second mechanism for phototropic curvature.

There are two further points in regard to photoinactivation. The first is that in the light-sensitive sporangiophores of certain fungi, Phycomyces and Pilobolus, the curvature also follows the carotene absorption (52, 58) and a small part at least of the auxin present is auxin a (172). These facts and the presence of carotene, demonstrated by Bünning (52) indicate that here also curvatures might be due to photoinactivation of auxin a lactone sensitized by carotene. Indeed, Kögl and Verkaaik (172) have no hesitation in drawing this conclusion, although undoubtedly most of the auxin of Phycomyces is indoleacetic acid, as was shown first by the diffusion constant determinations of Heyn (134). Furthermore, we have as yet no evidence that the growth of fungal hyphae is controlled by auxin. Hence this explanation for phototropic curvature in the fungi needs far more support.

* The "quantum yield" is stated to be very high—of the order of a million or more (170).
The second is that, in green plants exposed to the relatively high intensities of daylight, even indoleacetic acid produces less growth than in the dark, as shown by Thimann and Skoog (331). Elongation of all plant stems is, of course, reduced by bright light, and indoleacetic acid, as we have seen above, occurs widely as an auxin. As yet, there is not much quantitative information known about the photoinactivation of this substance, though in solution it does suffer a rather slow light-accelerated decomposition (Algeus, (5)).\(^7\) In crude plant extracts, which contain traces of carotene, it is rapidly inactivated by sunlight (187), and the same is true when indoleacetic acid is dissolved in agar. It is therefore entirely possible that phototropisin may be mediated by indoleacetic acid and is not, as formerly supposed, dependent on auxin a.

Finally the effect of light on auxin synthesis must be mentioned. All plants studied form more auxin in light than in dark (213,331), and on placing in complete darkness auxin rapidly disappears (see discussions in Went and Thimann, 360, Chapter 4, and in Boysen-Jensen, 46, Chapter 4. Oppenoorth (225) has, however, found that an increased synthesis appears within a few minutes after illumination of coleoptiles with moderately high intensities (3000–26000 ergs/cm.\(^2\)), and considers that the negative curvature and the second positive curvature are largely due to differences in auxin synthesis on the two sides. The increased auxin produced, insofar as it is auxin a, will of course equilibrate with its lactone and then be inactivated by light, and no doubt under long exposure, or continuous illumination, the two processes will keep pace. On the other hand, the increase may well be due to indoleacetic acid, for Larsen (187) found that when etiolated seedlings are exposed to light the (presumptive) indoleacetaldehyde decreases and acid auxin increases. This simple oxidation might account for such a rapid rate of formation of auxin.

A number of plants, particularly among the grasses, grow prostrate in the field, and Langham (185) has shown that in many of them this behavior is due to negative phototropism in sunlight, while in weaker light intensities they show normal positive phototropism. In connection with Asana's work mentioned above, an auxin analysis of these would be very valuable. It is important to note that "laziness" may thus be due to interference either with geotropism or phototropism (see p. 31).

C. OTHER TROPISMS

The geotropism of roots seems to agree with the Cholodny-Went theory. Root elongation is inhibited by auxin, except in the very lowest

\(^7\) The paper of Algeus contains an excellent discussion of the effect of auxin on unicellular algae.
concentrations (Section VII, B), and correspondingly there is good evidence that when roots are placed horizontal auxin accumulates on the lower side, reducing growth there and thus causing downward (positive) curvature. Traumatotropism, or curvature toward a wound, is due to two factors: the wound interferes with the transport of auxin, and enzymes set free by the killed cells rapidly inactivate auxin by oxidation. Both processes act in the same direction, i.e., to cause less growth on the wounded side. Other tropisms have been as yet insufficiently studied. A fuller discussion of tropisms will be found in ref. 360 (Chap. 10).

VI. Root Formation

The formation of roots on pieces of stem or “cuttings” was studied by early physiologists as a parallel case to the regeneration of organs in invertebrates. However, while the problems of regeneration are almost as obscure now as they were at the turn of the century, the nature of root formation has been considerably elucidated, mainly through the discovery of the role of auxin.

A. AUXIN AS A ROOT-FORMING HORMONE

The idea of an internal factor or hormone which controls rooting was first brought out by van der Lek (191), who showed that, when preformed root initials are not present, new roots are formed strictly at the base of a stem section; buds on the stem promote formation of roots below them and if the cortex below the bud is removed this effect is prevented. Thus he postulated a root-forming hormone produced by buds and travelling downward in the phloem (see Section IV). Following his work on auxin in the coleoptile, Went (349) showed that a diffusate from leaves, applied to the apex of a cutting, increased the number of roots formed, and Bouillenne and Went (40) then found that diastase and rice polishings extract were effective. These workers also found that application of sugar increases the number of roots formed, and they distinguished between its nutritive effect and the effect of the hormone, which is transported in a polar direction from apex to base only. The distinction between nutrients, stored in cotyledons etc., and special root-forming substances was also brought out by Némeé (215), whose ideas, developed independently, are similar to those of Bouillenne and Went in some respects.

Using the standard test of Went (350) with stem sections from etiolated pea seedlings, Thimann and Went (335) began the isolation of the root-forming hormone but soon found that the richest sources were materials like \textit{Rhizopus} medium and urine extracts (see p. 16) which
were rich in auxin; the root-forming activity accompanied the auxin activity through extraction with various solvents and all purification stages, and the chemical properties of the two hormones appeared to be identical. The identity was finally proved a few months later in two laboratories when synthetic indoleacetic acid was shown to have high activity for root formation on pea stems (Thimann and Koepfl, 323) and purified auxin a on *Tradescantia* stems (Kögl, 158); the latter plant material had just previously been shown to produce roots when treated with extracts of urine or pollen by Laibach, Müller, and Schafer (183) (see also 181).

The discovery that root formation on cuttings is induced by auxin, and the availability of synthetic auxins, have led to a vast amount of work on the application of this technique in horticultural practice. The rooting of cuttings is one of the main practical methods of propagation, of course, and much of the literature deals with conditions and concentrations of auxin most suitable for particular plants. An excellent review and a long table of results arranged by plant species and variety has been published by Pearse (240) and another long group of tables by Mitchell and Rice (205); a still more complete listing has just appeared (317a).

**B. Substances Active**

In general, all substances which have growth-promoting activity in one of the standard tests (see Section II) appear to be active in root formation. After indoleacetic acid and auxin a and b had been shown to be active, indolepropionic acid very weakly so, and indolecarboxylic acid quite inactive (323), Zimmerman and Wilcoxon (376) added α-naphthaleneacetic, indolebutyric, phenylacetic and fluoreneacetic acids, in approximately that order of effectiveness; Thimann (311) added indeneacetic and coumarane-2-acetic acids and showed that these two substances are poorly transported, but are fully active when applied to the base of the internode where the roots were produced. There is some uncertainty with phenylacetic acid, which appears to have no true root-forming activity and yet to be an auxin in other respects (354). Phenoxycarboxyacetic acids and their chlorinated derivatives, also naphthylacetamide, naphthylmethylsulfonic acid and 4-methylthiazole-5-acetic acid (339) are all active. The esters of some of these are almost as active as the acids, and being volatile can be applied to the whole plant in vapor form. Veldstra (339) has tabulated the relative activities of a great many substances for root formation.

**C. Interactions between Factors**

It is a peculiar fact that the combination of two auxins will sometimes produce more roots per cutting than one acting alone. This was first
shown for the combination of auxin a with indoleacetic acid (360, p. 195) and later for indoleacetic with naphthaleneacetic acid (9), for indoleacetic with phenylacetic acid (354), and for indolebutyric with naphthaleneacetic acid (139). Such effects are hard to explain, since it seems unlikely that each auxin can exert a fundamentally different effect and that these can then be summated. In the *Avena* test a weak auxin may actually inhibit the action of a stronger one (272a). It might be, of course, that certain cells or tissues enzymically destroy one auxin rather than another so that a single auxin cannot be effective on all tissues. Went (354) considers that root formation involves two processes, the first of which can be carried out by substances which are not necessarily auxin-active ("hemiauxins") while the second requires a true auxin; his experiments used successive treatments rather than mixtures.

The combined action of auxin and nutrients is more readily understood, for the formation of roots and their subsequent growth involves the laying down of cell walls and synthesis of protoplasm. Treatment with sugar, particularly with etiolated cuttings deprived of food reserves, often promotes rooting (40,350); but even woody cuttings (83,236,321, 322) are often benefited. Since cuttings are essentially starved during the ordinary process of rooting in the nursery bench, other nutrients are sometimes also effective. Complete nutrient solutions (21,107,325) may be used, but the calcium and magnesium may have inhibiting effects (325), and it seems rather that the principal constituents needed are nitrogenous, especially nitrate or ammonium, and adenine or other purines (77,236,325). The supply of organic nitrogen and of carbohydrates probably accounts for the favorable effect of leaves on cuttings, which is often proportional to the number of leaves present (144,248); indeed the effect of the leaves can be duplicated by a suitable combination of sucrose and nitrogen (236).

The growth of isolated roots in culture solutions *in vitro* is dependent upon thiamin (see following chapter), and while it might be thought that the minute amounts of thiamin needed for root growth on cuttings could be supplied by the stem, nevertheless thiamin does promote rooting of some cuttings (322,344) or subsequent growth after rooting (240). Other members of the vitamin B complex may be mentioned; biotin has a large effect on etiolated pea cuttings in auxin plus sugar (360, Chapter 11) which has not been reported for other plants, while nicotinic acid and choline (236,325) are also favorable. The role of an additional hormone-like substance, "rhizocaline," will be discussed in Chapter III.

D. ANATOMICAL STUDIES

The nineteenth century botanists, such as van Tieghem, were much concerned with the specific tissues from which roots arose. However,
the auxin work appears to show that root initials may be produced in almost any living tissue. They have been reported in epidermis, pericycle, endodermis, cortical parenchyma, and even in pith, particularly by Dorn (79), Kraus et al. (175), and Hamner and Kraus (129). In this sense plant tissues approach the “totipotency” of the animal embryologists. In line with the older views, however, roots do seem to arise more frequently from the pericycle than elsewhere (128).

E. METHODS OF TREATMENT

Root formation on cuttings can, of course, be induced by application of auxin at the apical end, its polarity of transport leading to rapid accumulation at the base. However, as mentioned in Section IV, the capacity for transport is limited so that, when high concentrations are applied to the apex, roots will be formed there also. Conversely, when high concentrations are applied to the base, roots are formed there only. Since many active substances are only poorly transported also, the logical procedure is to apply to the base. Concentrations from 0.25 mg./l. for sensitive herbs up to 200 mg./l. for resistant woody plants, applied for 24 hours to the base, are used in practice. A few seconds’ dip in highly concentrated (several grams per liter) alcoholic solutions is a practical alternative. The cuttings may instead be dipped in talc containing the auxin, enough adhering to the moist surface for effective action. Auxin may also be applied in lanolin paste almost anywhere on the cutting; this application is sometimes made a few days before the cutting is removed from the plant. Removal of epidermis, or even of the whole cortex, or splitting of the cuttings at the base, greatly facilitates auxin uptake in some species (e.g., Hubert et al., 145). The resulting increase in rooting may, however, be partly due to the wound stimulus. Uptake of the solution is favored by high transpiration or by partial drying of the cuttings beforehand (335a).

VII. PHENOMENA OF INHIBITION AND TOXICITY

One of the most curious features of the physiology of the auxins is that, while they promote so many growth processes, they also have growth-inhibiting effects. Two of the most marked of these are the inhibitions exerted on the development of buds and on the elongation of roots. The inhibition of the development of an abscission layer at the base of petioles and fruitstalks has many features in common with bud inhibition. Because the subject has been extensively reviewed (316) and because more recent work has thrown little fundamental light on the phenomena, a brief recapitulation here will suffice. In addition the general toxicity of the auxins, a subject with no direct bearing on the
normal hormone physiology of plants, will be discussed briefly because of its important applications to agriculture.

A. BUD INHIBITION

1. The Facts

In dicotyledonous plants the stem apex is a terminal bud. This bud normally produces auxin, mainly from the young developing leaves in it (12,17,331; see also 279), but also to some extent from the stem apex itself (101), and this auxin promotes the development of the stem immediately below it. However, the same auxin also prevents the development of lateral buds lower down on the stem, thus allowing the terminal bud to retain its "apical dominance." When the terminal bud is removed, as in pruning, one or more lateral buds (usually those in the most apical axils remaining) begin to develop; in so doing they also begin to produce auxin, which in turn inhibits the buds still lower down. If, after removal of the terminal bud, auxin is applied in its place, the lateral buds remain inhibited.

The first demonstration that bud inhibition is due to a diffusible substance was made by Snow (278), who showed that the inhibiting influence coming from the terminal bud in *Vicia faba* could cross a discontinuity of tissue; this experiment corresponds with those of Boysen-Jensen and Paál for the promotion of growth (see Section I). Eight years later Thimann and Skoog confirmed Snow's finding and identified the inhibiting influence with auxin, which at first (330,331) was obtained from *Rhizopus*, and later (273) with pure indoleacetic acid and auxin b. Laibach also showed that an inhibiting substance diffused from pollen (180). Confirmation with numerous different plants soon followed (73,81,101,136,337). The concentrations needed for inhibition, though somewhat higher than for growth, are entirely physiological and not toxic, for lateral buds which have been inhibited in this way resume their growth when the auxin source is removed (331). Several different natural and synthetic auxins have been shown to be effective (117,136, 176,273,311). It should be mentioned that leaves also exert an inhibition, though to a lesser extent than the terminal bud, as was shown earlier by Dostál (80).

2. Mechanisms

The way in which the inhibition is brought about is far from clear. The many hypotheses have been reviewed by Snow (283) and Thimann (316). At first it was thought that the auxin at the apex (either produced naturally by the bud, or applied artificially after decapitation) in some
way diverted to itself substances necessary for bud growth and thus starved the lateral buds (211,354; see also the discussion in Section V of the following chapter). This is similar to the view of Goebel and other older botanists who considered that a growing apical bud maintained its dominance by using up the available nutrients. A modification of this view is that of Ferman (85), who suggested that the growing bud draws to itself the supply of auxin precursor, so that the laterals are unable to produce auxin. This is supported by the undoubted fact that inhibited buds produce less auxin than do growing buds (331) and, though the evidence is not quite consistent, they also appear to contain less total extractable auxin than growing buds (85,228). In other words, there is some reason to think that the inhibition is exerted not so much on the growth of the bud as on its ability to produce auxin.

However, it now seems clear that inhibition cannot be primarily an indirect effect due to the diversion of materials away from the bud, since application of auxin directly to the lateral buds, either *in situ* on the stem, as in the experiments of Plch (241) and Thimann (314), or isolated and growing in nutrient solution, as by Skoog (272), causes clear-cut inhibition of their growth. Also in small fragments of plant tissue in culture, particularly root tissues, auxins such as naphthaleneacetic acid strongly inhibit the development of buds (96,98). In slices of potato tuber the local application of auxin inhibits bud development without producing any corresponding growth elsewhere (81,202a). Exposure of whole potatoes to auxins in vapor form (*i.e.*, methyl esters of the acids) causes inhibition of all the buds (123). In none of these cases would it seem that the effect can rest on movement of materials elsewhere; the effect is primarily local.

It appears that the influence of auxin on different organs is represented by a series of optimum curves, intermediate concentrations promoting growth and higher concentrations inhibiting it (45,314), as shown in Fig. 7 (p. 45). Thus the concentrations causing stem growth would be high enough to inhibit bud development. This general theory receives support from the numerous effects of auxin in Gautheret's cultures of various organs (96; see Section VIII, A), and additional considerations which may help to explain it have been advanced by Skoog *et al.* (272a); against it is the lack of any cambial activity in inhibited buds (280) although auxin is known to stimulate the cambium (see Section VIII). A peculiar and unexplained fact is recorded by Castan (57), namely, that, if high auxin concentration is applied to the intact terminal bud, it loses its power of inhibiting the lateral buds below it.

The problem is made more complicated by the direction in which inhibition is exerted. Since auxin moves polarly from apex to base,
inhibition should be only exerted on buds morphologically below, i.e., basal to, the source of auxin. Although this in general is strictly the case, there are exceptions in which buds are inhibited above the point of auxin application (283,285), and a parallel has been suggested with certain upward inhibitions of stem elongation, studied by Pohl (243), Le Fanu (190), and Mitchell and Martin (204). The phenomena of geotropism, however, provide clear agreement with expectation; here the auxin is known to be diverted to the lower side of stems by gravity, so that we should expect to find that in horizontal stems the buds on the lower side are inhibited; this was observed as long ago as 1917 by Loeb and has been confirmed in different plants by many workers (73,237,249,272).

One type of phenomenon which might have significance for bud inhibition has not yet been brought into the picture. Many workers have found evidence for growth-inhibiting material in plant tissues, particularly in ether extracts thereof. Köckemann (154,155) extracted such material from fruits, demonstrating its effect by inhibiting the germination of seeds. This so-called "blastokolin" was investigated by Kuhn et al. (177), who extracted an oil from Sorbus fruits which strongly inhibited seed germination, and demonstrated that parasorbic acid had similar effects. Other substances having an unsaturated lactone structure (340), including coumarin, act in the same way. Moreover, Voss (342) extracted from corn, and Larsen (186) from tomatoes, material which inhibits growth of the Avena coleoptile. Linser (199) made similar extracts from lilac leaves and showed that they also inhibit the formation of roots. Juel (152), in an extension of Larsen's work, utilized his assay method of mixing the inhibiting extract with known concentrations of auxin in agar and using the Avena test on the mixture. She showed that the inhibition is not due to auxin inactivation, and that it is exerted also on root growth, which itself is inhibited by auxin (see pp. 43–46). Hence the extracted material is not simply an antiauxin, but an inhibitor of the growth of both shoots and roots. Similar experiments have been carried out with sugar cane nodes, from which the inhibitor is liberated by hot water (237,237a). The dormancy of potato buds has been shown by Hemberg (131a) to be due to an inhibitor present in large amounts in the periderm, and disappearing slowly as the tubers mature. The auxin content does not change during dormancy but increases shortly before sprouting.

More suggestive still is the inhibitor of Stewart et al. (297,299), which produces a marked positive curvature (i.e., toward the block) in the Avena test. This substance was partially purified and shown to yield an auxin—most probably indoleacetic acid—on alkaline hydrolysis. If it could be shown that lateral buds have the property of producing this
inhibitor directly from auxin, a mechanism of bud inhibition would be at hand. Although as yet there is no such direct evidence, the scheme advanced by Skoog et al. (272a) gives a very plausible rationale for this. Furthermore, Snow (286) has brought forward independent evidence that bud inhibition is due to a special inhibiting hormone in some way produced by auxin. This concept has recently been discussed by Skoog (274a).

The situation can be summed up by saying that most of the data point to bud inhibition as due to auxin directly, with the mechanism probably involving the formation of an inhibitor by or under the influence of auxin. The possibility is not excluded, however, that other substances necessary for growth may in some way play a part.

3. General Significance

The inhibition of one bud by another is a phenomenon of very wide occurrence and has a broad influence on general morphology. In tubers, for instance, development of a bud at the apical end leads to inhibition of others, but ringing, or physical isolation of these buds, allows the lower buds to develop (81,131,202a). The auxin is presumably carried from one bud to another through the cortex. Auxin application, either as paste to the outer cortex or as vapor to the whole tuber, maintains the buds in the inhibited state, and this is now being used on a large scale in the storage of potatoes, with methylnaphthalene acetate. It is of interest that such auxin-inhibited buds resemble normal dormant buds in that they are stimulated to sprout by ethylene chlorhydrin (123,202a). This treatment greatly increases the rate of auxin destruction, thus releasing the buds from inhibition; when growth begins again the terminal bud soon re-establishes its inhibition of the laterals, again through the auxin mechanism (202a). It is not free auxin itself, however, but a specific inhibitor (131a) which is responsible for the absence of bud development during the dormant period.

In general the tall, rapidly growing single-shoot type of plant, which presumably produces and transports auxin efficiently, has few lateral branches, while shorter dwarf or stunted forms typically become bushy with numerous laterals or tillers. Auxin relations of this sort have been studied by van Overbeek (227,230) and Delisle (73) but much still remains to be done. Young leaves, since they are potent sources of auxin, exert a powerful inhibition (279) but mature leaves also inhibit in some plants (80). In guayule, a desert composite grown for its rubber content, the mature leaves actually inhibit the buds in their axils more powerfully than does the terminal bud (277). Indeed, a single leaf can inhibit the lateral buds all the way down the stem, a most unusual behav-
ior, which may well repay closer study. In *Solidago* plants in the rosette stage, each leaf inhibits somewhat the growth and development of the next, a phenomenon presumably parallel to that of bud inhibition (101).

In the ferns, Albaum (1) has brought to light a parallel situation; the heart-shaped prothallia respond to the removal of their growing apex by formation of a new outgrowth (of the same shape as the indented area which they replace), and this "regeneration" can be inhibited by applying auxin in lanolin. Similarly, if the young sporophyte which develops later out of the prothallium is removed, another grows in its place, while application of auxin to the cut stump prevents this. These phenomena are thus quite parallel to the inhibition of buds, although buds as such are not involved. Doubtless Nature has provided many similar variations on the same theme.

**B. Root Inhibition**

Besides simple growth promotion, the first additional effect of auxin to be discovered was the inhibition of the elongation of roots. This was when Nielsen (217) extracted a crude auxin from cultures of the fungus *Rhizopus suinus* and showed that it promoted growth of the coleoptile but inhibited that of the root. The experiments were repeated and extended by Boysen-Jensen (44) and Navez (214) and finally done with pure auxins by Kögl, Haagen Smit, and Erxleben. The technique is simply to immerse the roots of young seedlings in serial dilutions of the auxin and measure elongation with a millimeter scale. There is some thickening, but this is not, as was first thought, sufficient to compensate for the decrease in length; the auxin therefore produces a large total decrease in root weight (312). The inhibition in length is roughly proportional to the logarithm of the concentration, so that the effect has been used as a simple auxin assay by Lane (184) and Bonner and Koepfli (34). Control of pH is essential (202), since auxin enters tissues much more readily in the free acid form than as an ionized salt (4,326). The activities of a number of substances have been compared in this way (92,184,202,311, especially 34) and it appears that, in general, compounds which have auxin activity as measured by growth promotion also cause root inhibition; if inactive in the *Avena* or pea test they are inactive in inhibiting roots. The inactivity of indolecarboxylic, α,α-dimethyltoluic and trans-cinnamic acids (34) is of particular interest in connection with the relation between structure and activity discussed in Section III, C. Recently Thompson et al. (336) have published this as a new method, and tested 1060 compounds with it. Of these, the most active were: 2,4-dichlorophenoxyacetic acid, its anhydride, sulfoanilide, and certain esters;
2-methyl-4-chlorophenoxyacetic acid, its anhydride, amide, some esters, and other derivatives; 2-bromo-4-chlorophenoxyacetic acid; and 2-methyl-4-fluorophenoxyacetic acid. The first-mentioned is highly active in the curvature of slit pea stems (see Section II, D), though it gives only minute curvatures on *Avena* coleoptiles. Doubtless all these substances will be found to show growth-promoting activity on one or other of the standard growth-promoting auxin assays.

Of course, an inhibition is less specific than a growth promotion, and many compounds have some inhibiting effect in relatively high concentrations. For this reason the inhibition of germination, studied by soaking seeds in solution and termed the "blastokolin" test (see Section A above) may not be very specific; it appears, however, to have no relation to the inhibition of root elongation by auxin. For instance, the ether-soluble growth inhibitor of tomatoes inhibits both root and shoot growth (152). It is well known, too, that colchicine inhibits root elongation and causes characteristic swellings just proximal to the root tip (see, e.g., 82,192,201). It is perhaps remarkable that the changes in electric potential differences along the root which are caused by colchicine treatment are very similar to those caused by indoleacetic acid (338). This does not, of course, necessarily mean that, as Umrath and Weber (338) suggest, colchicine produces its effect by "activating" auxin in the root, for its effect on mitosis is far stronger than that of auxin. However, it is at least suggestive that the swellings induced by auxin in roots were shown by Levan (193) to contain many polyploid cells.

In contrast to the inhibition, extremely low auxin concentrations cause slight acceleration of root growth. This was discovered independently by a number of workers in 1936 (7,8,54,84,86,99,312); only Jost and Reiss (151) could find no acceleration. The effects are small but real; indoleacetic acid at $10^{-9}M$ causes about 30% acceleration. The response of roots to auxin is thus given by an optimum curve with its peak at excessively low auxin concentrations, as shown in Fig. 7. Also, if the inhibition is not too great, it is accompanied by the formation of lateral roots, i.e., by branching (151,312,372). The same effect results from decapitation of the root tip. However, such branching is not simply due to the inhibition of the tip growth, but is directly caused by auxin, because, as shown by Thimann (311), when auxin is applied to the base of the stem of *Pisum*, it slightly accelerates the growth rate of the main root, but still promotes the formation of laterals.

Short exposure of roots to auxin causes a temporary inhibition followed by a stimulation, which may lead to a general stimulation of growth of the entire plant (92,324). This "after-effect" is probably the cause
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of the accelerated growth of "hormonized" seeds first reported by Cholodny with oats in 1936, and discussed further on p. 54. The duration of the inhibition is proportional to the time of exposure to the auxin, and Gast (92) has shown that the amount of stimulation which follows is roughly proportional to the amount of inhibition. A detailed analysis of the phenomena of root elongation will be found in the papers of Burström (54). He divides the process into a phase of increasing elasticity, which is accelerated by auxin, and one of decreasing elasticity (during which most of the growth takes place), which is inhibited by auxin.

The effect of auxin in inhibiting root elongation acquired special interest as an explanation of the geotropism of roots (see 360, Chapter 9). This geotropism, which is positive, i.e. toward gravity, would thus be due to the accumulation of auxin on the lower side as in shoots, but with the difference that the auxin would cause greater inhibition on the lower side. This was the original Cholodny-Went theory of geotropism, but it has never been really rigidly established. While all experiments point in this direction, the closeness of the growing zone to the tip in many roots has made it extremely hard to obtain clear-cut growth responses after decapitation. The production of auxin by the root tip has also been hard to establish, in spite of many extraction and diffusion experiments (see especially 44,45,86,246,247,267). To sum up briefly many contradictory facts and interpretations (discussed by Fiedler, 86, and Thimann, 316), it appears clear now that small amounts of auxin are in fact regularly produced in the root tip provided it is adequately nourished (235,267). If this is to be enough so that its geotropic accumulation on
the lower side would account for positive curvature, it should also be
enough to cause at least slight growth inhibition when the root is in the
vertical position. In other words decapitation should cause slight
acceleration of root growth. Some investigators have indeed found this
effect, but agreement is not complete, perhaps due to the morphological
difficulty mentioned above, which makes the length of the tip cut off
extremely critical. It should be noted, too, that exposure to light
increases the auxin content of isolated roots (267) and correspondingly
inhibits elongation (253). Differences in lighting may thus also account
for lack of agreement among different investigators.

Since high auxin concentrations also inhibit elongation of stems it
might be supposed that stems supplied with considerably more auxin
than they receive under physiological conditions should show positive,
*i.e.*, downward, geotropism. This has been claimed, indeed, by Geiger-
Huber and Huber (100) with mustard seedlings, but it is more probable
that the downward curvature reported is not due to growth, but merely
to plastic sagging, since Burkholder (53) has shown that similar down-
ward curvatures are prevented by balancing the weight of the shoot.

C. INHIBITION OF ABSCISSION

The falling of leaves and mature fruits is due to the formation of an
"abscission layer" of cells across the base of the petiole or fruitstalk,
and to the separation of the walls of these cells from one another. In
experiments with *Coleus*, Laibach (180) found that this abscission is pre-
vented by applying orchid pollinia to the petiole. The phenomenon was
discovered independently by La Rue (188) and shown to be produced by
several pollens and leaf diffusates and also by pure auxin (indoleacetic
acid). *Coleus* is convenient for these experiments because the petioles
fall quickly when the blades are cut off; *Ricinus* and *Bryophyllum* behave
similarly. The reaction is simple, and by its means Gardner and Cooper
(89) have compared the activity of nine auxins and shown that 156 other
compounds without auxin activity do not delay abscission.

The interest in this phenomenon lies primarily in its application to
fruitstalks, which often absciss before the fruit is completely mature.
Gardner and Marth (91) and Hoffman *et al.* (141) showed that the pre-
mature dropping of apples can be conveniently delayed by auxin treat-
ment. Spraying or dusting with auxin in early September will delay fruit
drop at least two weeks. This procedure is now widely used by orchard-
ists; directions for its use have been given by many experiment stations.

Falling of the needles of evergreens, at least in *Tsuga* (335a) and
*Taxus* (81a) is also delayed by auxin; in *Taxus* a concentrated nutrient
solution acts in the same way.
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D. GENERAL TOXICITY

It has been known for many years that high auxin concentrations are toxic. This was first noted in experiments with plant parts immersed in solutions for growth measurements (30,326,334), with whole plants treated with auxin solutions (106), and with cuttings treated with auxin solutions at the base (by many workers, see 360, p. 204). In concentrations just below the toxic level, growth inhibition commonly occurs (see discussion in 316) and inhibitions may be caused above a local application of auxin to the stem (190,207,242,282). Further, as discussed above, root growth is powerfully inhibited by auxins. The toxic effects, as opposed to mere inhibition, have been recently put to practical use. In parallel experiments in the United States and England, it has been shown that simple spraying with relatively high concentrations of auxins (about 1000 mg./l.) will kill many dicotyledonous plants. The most effective substances are those which are of high auxin activity and stable against soil microorganisms, particularly 2,4-dichlorophenoxyacetic acid and related compounds (22,27,127,275,276,308,336,345). Aqueous sprays of the free acid or its esters or salts appear to be the most effective. Because the grasses and cereals are relatively insensitive, it is possible to exert what the English workers call "selective herbicidal activity," and destroy weeds in standing cereal crops. This application is of very great agricultural importance and is already being used to eliminate such pests as ragweed, bindweed, and water hyacinth. No attempt will be made here to discuss or even list the flood of papers on this topic in recent horticultural literature. A recent review has been given by van Overbeek (234b).

The exact nature of the toxicity of auxins is not clear. The killing of whole plants in soil may rest in small part on root inhibition, but usually involves complete rotting of the roots and rapid dying of the leaves. Furthermore, toxicity is exerted on isolated stem or coleoptile sections in solution and indeed at concentrations as low as 50 mg./l. Such objects show a clear optimum curve in their auxin response. It is probably significant that many toxic substances cause stimulation at low concentrations, inhibition or toxicity at high. Examples are the heavy metals, cyanide, 2,4-dinitrophenol, and iodoacetate. However, in none of these instances has the change of sign of the effect been satisfactorily explained. In popular literature it has been stated that the auxin weed killers cause plants to "grow themselves to death," but there is little basis for this statement.

* Went (private communication) has, however, found that some substances inactive as true auxins are effective as weed killers.
VIII. Other Actions of Auxin

A. CELL DIVISION

1. Tissue Cultures

The phenomena of cell division in isolated fragments of plant tissue were first studied some forty years ago by Haberlandt in his classical but unsuccessful attempts to obtain plant tissue cultures. The conditions leading to cell division in plant tissues are many and varied, but one of the main contributions of the work on tissue culture was to direct attention to the role of hormonal factors in the process. The action of wound hormones and their possible interrelation with auxins in promoting cell division will be taken up in Chapter III; we will deal here only with the role of the auxins themselves in cell division.

It is characteristic of roots that they grow well in culture media, with cell division keeping pace in a normal manner with cell enlargement, and without the necessity of adding any auxin. There is no evidence that roots need any supply of auxin for their growth, though it is possible that they do produce small amounts of an auxin and that this suffices for their needs. Much of the auxin in root tips disappears rapidly soon after separation from the plant (86) but small amounts remain; it is highly probable, though not rigidly proved, that there is a slow production of auxin by the tip even on a mineral medium (212, 232, 235). The total auxin of roots, like that of many other tissues, is extractable with ether only very slowly (332); with Avena roots about three weeks of continual extractions are needed to reach a 75% yield. The tumor cultures of White (363) also grow slowly without added auxin, but they definitely produce small amounts of it during growth (332).

In other instances cell multiplication appears to depend markedly on the presence of auxin. This is well exemplified in tissue cultures. While slices of carrot will develop for many transfers (with cell division) in a mineral medium containing only salts, sugar, and a source of nitrogen, as was shown almost simultaneously by Nobécourt (218, 219) and Gautheret (93, 94) in 1937–1938, their cell division and growth are very greatly promoted by auxin at 1 mg./l. Indeed, Nobécourt considers auxin essential for the carrot, since its growth invariably stops after some months unless auxin is added. This suggests either a very slow synthesis, or else a remarkable persistence of auxin in the tissue. The tissue of Jerusalem artichoke (Helianthus tuberosus) develops only if auxin is added; indoleacetic or naphthaleneacetic acid at 0.1 to 1.0 mg./l. are about equally effective. For carrots, indolepropionic acid is ineffective (221). In such material the auxin behaves therefore as a cell division-
inducing substance. Some cultures have been kept going, in presence of traces of auxin, for over four years (Fig. 8). Gautheret (95) points out that the new tissue formed in culture fragments is proportional to the amount of surface exposed, which might suggest that wound hormones liberated at the cut surfaces also play a part (see Chapter III, Section 1). The differentiation in the cultures seems not to be auxin controlled, since it takes place also in carrot, kohlrabi, and endive cultures, which do not, at least at first, require added auxin (97). Such conclusions, however, are uncertain until the formation of auxin in these cultures is examined.

![Fig. 8.—Culture of endive tissue which has been maintained for over 4 years in presence of traces of auxin. The fragment shown has grown for 28 days after the 25th transfer. (From Gautheret, 98.)](image)

Thiamin is certainly synthesized by the carrot cultures of Nobécourt (220).

The concentration series in the action of auxin here is of interest (96). With carrot, endive, and Jerusalem artichoke, increasing concentrations of naphthaleneacetic acid produce, in order: (a) cambium stimulation with callus formation; (b) root formation; (c) bud inhibition; (d) an action on leaf growth; (e) isodiametric growth of cells, causing general swelling. The last is typical of high auxin concentrations in many plants (see below).

2. Cambium

A clear-cut promotion of cell division is produced in the cambium of many plants by treatment with auxin. This was first demonstrated by
Snow (281), who had previously shown that some diffusible substance causes activation of the cambium in grafting, and indeed had been fore-shadowed by Jost forty years earlier. The amount of indoleacetic acid necessary to cause cell divisions in the cambium of sunflower hypocotyls was shown to be comparable with the amount normally produced by buds and young leaves, as determined by Thimann and Skoog (331) in diffusion experiments. Thus cambial activation by auxin is a normal plant process (173a, 281, 292, 293a). The activation which travels from the opening buds downward throughout the stem in the springtime is hence due in the main to auxin. Vigorous cambial activation, i.e., cell division, was shown to result from auxin treatment of twigs of willow and poplar by Söding (291), who also showed (293) that the auxin travels polarly from apex to base in the twigs, mainly in the cambium itself. Before the buds open, organic matter migrates to them in considerable amount (55) and auxin begins to be liberated thereafter, as actual opening proceeds. That this auxin moves downward in a wave lasting only a few weeks was made clear by Zimmerman (377); this movement is followed closely by division of the cambium. The close time relations were shown clearly in apple by Avery et al. (16), who compared sections of the wood at different times in the spring, and different distances from the bud, with Avena test determinations of the auxin coming from the buds. It is characteristic of experiments with applied auxin that the cambial activation is generally limited to a few centimeters below the point of application, while the natural stimulus moves to ground level or even into the roots (292); it is important, therefore, that if the application is made within a limited period in the spring the resulting cambial activation can also travel great distances (105). Apparently, however, the active substance in cambium is not auxin alone, for Söding (293a) finds that cambium-stimulating preparations obtained from cambium itself are more active than the corresponding concentration of indoleacetic acid (see Chap. III, Section 1). Söding found that more auxin diffuses from the cambium than from any other tissue, in woody stems, and this has been confirmed for a number of tropical plants by Kramer and Silberschmidt (173a). There is, of course, no reason to believe that the auxin is responsible for differentiation into xylem and phloem.

When trees grow in a leaning position the wood formed on the underside is reddish and of characteristic morphology; this was described in 1896 by R. Hartig and such wood termed "rotholz" or "redwood." Wershing and Bailey (362) were able to duplicate this in white pine seedlings by auxin application and it is likely, therefore, that the extra auxin accumulated geotropically on the lower side of the stem is responsible for the natural phenomenon. If this is true, the great excess of
auxin applied in Söding's experiments (291) should also have produced redwood, a point which deserves further anatomical study.

Application of auxin to woody twigs or cuttings also causes the formation of so-called callus, particularly at the basal cut surface (see 87, 182, and many others). The weight of callus so formed on poplar varies directly with the concentration of auxin applied (254), but again it falls off rapidly with increasing distance and reaches zero at about 3 cm. below the point of application.

3. Other Tissues

In Snow's experiments (281) only the cambium divided as a result of application of auxin at physiological concentrations, but later Kraus, Brown, and Hamner (175) and Hamner and Kraus (129) found the endodermis very reactive when the auxin concentrations were higher. In young bean stems, mature vacuolated cells of many tissues enlarged and divided, later forming many root initials. Tomatoes (37) and four o'clock (Mirabilis) (128) behaved similarly. It should also be pointed out that formation of root initials always involves very active cell division which often originates in the pericycle, but may occur in every living tissue from epidermis to pith (see Section VI above).

The first result of application of high auxin concentrations to young stems or hypocotyls is a very great swelling of the pith and cortical parenchyma (28,75,175,182). The same thing happens at the base of auxin-treated cuttings (71,301, and casual observation of many workers on root formation). In these swellings starch is rapidly hydrolyzed (21,203,208); then organic materials are transported to the swelling from adjoining parts of the stem (206,207,301); the cells, particularly of the cortical parenchyma, increase greatly in size, while those of the epidermis shorten (see especially Figs. 32 and 36 of Diehl et al., 75). Very large cells are also formed in tissue cultures exposed to auxin concentrations above 1 mg./l. (98, pp. 97–100). Cell division comes relatively late, usually after several days, and is seen in many tissues. It is of interest that the nuclei in such swellings reveal chromosome doubling; tetraploid and even octoploid cells are formed (74). Similar polyploidy occurs in the callus tissue growing on cut surfaces of the stem after auxin treatment (109), although it is not clear how far this is due to the auxin, since polyploid tissue occurs also in natural calluses. The auxin-induced swellings of roots contain nuclei with internal chromosome multiplication also (192).

4. Pathological Changes

Galls on stems, and nodules on the roots of legumes, both involve numerous and continued cell divisions. In the case of galls due to the
crown gall bacterium (*Phytomonas tumefaciens*), Link and Eggers (196) have shown that the infected tissues are very rich in auxin, and Brown and Gardner (51) and Link *et al.* (198) have produced gall-like growths by continued application of indoleacetic acid to the cut surface of a young bean plant after decapitation. Naphthaleneacetic acid and its amide can also produce gall-like swellings (176,203). However, in later stages of the growth of crown galls, neither auxin (252) nor even the bacteria (364) can be identified, so that an explanation based on auxin production by the bacteria cannot account for all the phenomena of crown gall. Indeed, secondary galls were produced by sterile inocula from the original galls by White and Braun (364), which indicates that the host cells have been permanently altered, as in animal cancer. This phenomenon was shown more strikingly by *in vitro* grafts of tumor tissue to sections of normal stems (255). In this work de Ropp shows (as the Wisconsin workers had done earlier) that crown galls on the intact plant in many respects behave as though they produce auxin, since they cause root formation, root thickening, and sometimes bud inhibition in adjacent normal tissues. However, the comparison is not perfect because in the grafts the only effect on the normal tissue is that of disorganized proliferation and roots are not formed, while in normal tissue proliferation occurs only at very high indoleacetic acid concentration and at all physiological levels roots are formed. He concludes that the diffusible "tumefacient factor" is probably not identical with auxin.

Nodules on legume roots are also very rich in auxin (194,195,313,315); unlike most auxin in plant tissues this material is wholly free and rapidly extractable (332). Since the invading rhizobia certainly form auxin in culture media (59,313), Thimann (313) proposed the following picture for nodule formation: the invading bacteria form considerable amounts of auxin, which causes cell division in the endodermis or pericycle. Such division would normally lead to the formation of a secondary root, but since the elongation of roots is strongly inhibited by auxin (see Section VII) the result is a more or less isodiametric swelling. Kraus (174), however, states that in nodule formation the first cell divisions occur in the cortex, so that the nodule is not strictly homologous with a lateral root.

### B. Formation of Fruits

As long ago as 1909 Fitting found that the swelling of the ovary of certain orchids, which normally follows pollination, can be brought about by applying extracts of the pollinia. Morita (210) later obtained similar results, and Laibach (179) showed that the active substance, both of orchid and of *Hibiscus* pollen, could be extracted with ether. Further,
the extract behaves like auxin and its effect can be duplicated with ether extracts of urine, etc. (180). Pollen of many plants contains an auxin active on Avena (112,309,335). Yasuda (368), using aqueous extracts of pollen, obtained quite large swellings of the ovaries of Solanum and also (369) almost normal-looking fruits of cucumber. Since these were formed without fertilization they were seedless or "parthenocarpic."

Final proof that this reaction is due to auxin was given by Gustafson (111), who produced mature seedless fruits of tomato and other plants by applying indoleacetic acid and other auxins, in lanolin paste, to the styles before fertilization could occur. Mature seedless pepper, crookneck squash, and even watermelon were produced by Wong (366), holly and strawberries by Gardner and Marth (90), pears by Sereskiü (268), and other fruits in the same way. For commercial use a mixture of seedless and fertilized fruits, with a total increase in the number of fruits set, is often sufficient.

The method of application has been the subject of considerable practical study. Gardner and Marth (90) used a water spray, Howlett (142,143) a lanolin-water emulsion, and Strong (300) a mixture of auxin with trigamine or morpholine applied to the entire flower bud cut off just above the ovary. Zimmerman and Hitchcock (372,373) obtained seedless fruits of holly by means of the vapors of auxin esters, and of tomatoes with an aerosol of auxin esters (373). Both these treatments were applied to the whole plant. To obtain completely seedless fruits, of course, the styles must be removed before the pollen tubes can have grown through, but Howlett (142,143) has shown that, at least in the tomato, pollination is often imperfect, so that for practical growers' purposes the flowers can be left intact and, after spraying, the growth of all fruits is promoted by the auxin treatment. Blossom end rot and bud inhibition often occur in sprayed fruit. A list of parthenocarpic fruits produced by auxin up to 1942, and also a list of the plants which produce them naturally, is given in the review of Gustafson (119).

The relative activity of different auxins for parthenocarpy, though not easy to determine accurately, seems to place the different substances about in the same order as for root formation, or perhaps for primary growth promotion (see Section III, C), but not in the same order as in the Avena test or the pea test. Gustafson (113,115,121) found α-naphthoxy-acetic and indolebutyric acids the most active, but later the di- and tri-chlorophenoxyacetic acids were found to be much more active (372,373). Such relative activities are doubtless determined, at least in part, by relative stability to plant enzymes under the long exposure involved in this type of experiment. Should the finding of Tang and Bonner, i.e., that the inactivating enzyme system in the pea is specific for indoleacetic
acid, be extended to the tomato and other plants, it would provide a good explanation for the relatively low activity of indoleacetic acid for parthenocarpy.

The mechanism of this phenomenon is not fully understood, but a tentative picture has been presented by Gustafson (114,120). The auxin introduced either by the pollen or by artificial application starts growth by cell enlargement in the ovary tissues. This, in fertilized fruit, leads to growth of the ovules themselves, and they then secrete auxin (their natural auxin content is high) in sufficient amount to cause continued growth of the ovary tissues. Plants which readily produce parthenocarpic fruit, such as the navel orange, contain somewhat more natural auxin in the ovary walls than other varieties of the same species which do not show parthenocarpy. It is this auxin in the ovary walls which then suffices for further growth after the first "shot" of auxin has initiated it. This concept is based on auxin determinations in various parts of fruits of different species and varieties, and their correlation with parthenocarpy or even (120) general fruitfulness; the data are, however, not wholly clear-cut and the picture may need extensive modification. In particular the concept that auxin secretion does not begin until growth has been started needs clarification. There are certain suggestive parallels here with the growth of buds, in which the initial stimulus is furnished not by auxin (which inhibits) but by other factors, but thereafter auxin production follows growth.

C. ROLE OF AUXIN IN SEED GERMINATION

It was first shown by Cholodny (62) that oat seeds treated with auxin show a subsequent stimulation of growth. This he compared to the effects of vernalization, in which the seeds are moistened and then kept cool for a long time; under such conditions auxin is set free within the endosperm in considerable quantities, by enzymic action (61,271,342). The nature of the precursor in the endosperm, which liberates the auxin, is discussed in Section III, and need not concern us here. The auxin set free in the endosperm does not, as it now appears, operate to produce vernalization, for Gregory and Purvis (110,245) have shown that the isolated embryo, freed from endosperm, can show normal vernalization, while Hatcher (130) finds no auxin in the rye embryo during germination at normal or vernalization temperature. The acceleration of growth following treatment of the seed with auxin is a purely vegetative phenomenon. Using indoleacetic acid, Thimann and Lane (324) showed that the inhibition of root growth which first appears after auxin treatment is later followed by an acceleration both of elongation and of branching, i.e., formation of secondary roots, and they ascribed the improved top
growth to this effect, which would lead to an increased total root system; indeed the roots of full-grown oat plants so treated showed a large increase in weight over controls. Amlong and Naundorf (9) obtained similar growth accelerations with many seeds, including sugar beets, which gave an increased yield of sugar per acre as a result. It is important that the stimulation of growth, although it may not be very large, lasts throughout the life of the plant, at least in some cases. However, several other workers (e.g., Barton, 20; Templeman and Marmoy, 307) have failed to obtain any appreciable effect from seed treatments, so that the conditions of treatment are apparently quite critical and need further analysis. Podešva (241a) reports good results with several vegetables.

IX. Mechanism of the Action

It will be clear from the preceding sections that the effects of auxin on plant cells are numerous. Growth by increase in size is the major and most direct effect, but stimulation of cell division, without increase in size, in the cambium, in root initials, and in fruit formation is at least as important. Clear-cut inhibitions of growth of buds, roots, and the abscission layer appear also to be direct effects. The action of auxin on the cell must therefore be a fundamental one, a kind of "master reaction." The consequences of the process may lead to growth, inhibition, etc., according to the supply of other factors and to the age and morphology of the tissues concerned.

A. Effects on Cell Wall

Before it was recognized that phenomena other than simple cell enlargement were involved, Heyn (132,133) and Söding (289,290) brought forward considerable evidence that the effect of auxin, at least in the coleoptile, was to increase the plasticity of the cell wall. The plant cell differs, of course, from that of the animal in its relatively rigid cellulose wall, which resists the osmotic tendency of external water to enter and thus holds the cell size in balance. Increased plasticity would decrease the pressure of the wall on the cell contents and thus allow water to enter osmotically, increasing the cell size. The evidence was obtained by applying known loads to the plasmolyzed coleoptile or other organs, and measuring the irreversible or plastic stretching which resulted (135). Another method is to plasmolyze the plants after they have produced a curvature in response to auxin; the decrease in curvature resulting is in the part which was purely elastic.

9 A full discussion of the early work, up to 1937, is given in Chapter VIII of Phyto-hormones (360), and by Heyn (135).
The plasticity of the coleoptile was found to decrease following decapitation and to increase again with the "regeneration of the physiological tip" after about 2.5 hours. Application of auxin in agar to coleoptiles, flowerstalks, or stems clearly increased the plasticity. Some of these experiments have been more recently repeated by Burkholder (53) with similar results. Also auxin in lanolin gave essentially the same effects (256). It is clear that it is the change in plasticity, not in elasticity, which parallels change in growth rate. This is particularly striking in roots, where auxin acts to increase the elasticity, whether it causes increase or decrease of the growth rate (54). The conception of growth which is involved is that the wall, after being made more plastic, is stretched by the entering water and then fixed in its stretched state by the interposition or apposition of new cellulose particles. Bonner's measurements (32) of the weight of the cell walls indicate that, when growth occurs at 2°C, the latter process lags behind; when it occurs at 25°, or in the presence of sugar, cell wall deposition exceeds growth and the weight per unit length increases. However, it seems that some minimal cell wall deposition must keep pace with extension.

A modification of the above view, according to which the auxin acts mainly on the pectic substances of the middle lamella, has been put forward by Ruge (256–258), with, however, insufficient experimental support. According to his data this pectic material, which is said also to contain hexosans and hexonic acids, swells in auxin and it is this swelling which leads to growth. To a lesser degree the swelling is also caused by acid pH, which is known to promote growth (31,311). Hydrolytic enzymes are also claimed to promote growth through hydrolysis of the pectin, although it has been known since the work of Seubert (269) that commercial enzyme preparations commonly contain some auxin.

A more extensive consideration of the effect of auxin on cell walls, based both on experiment and on theory, has been set forth by Diehl et al. (75). These workers believe the action is first exerted on the intermicellar substance, which is probably of the nature of a wax (367), and thereafter on the cellulose micelles themselves. The skeleton of the primary wall, according to the observations and concepts of Frey-Wyssling (88), consists of micelles of cellulose oriented (statistically) perpendicular to the axis of elongation. This skeleton has to be continuously modified to allow growth. Unpublished observations of the author and T. Kerr indicate that this takes place by a continual loosening and re-forming of the linkages between crisscrossed micelles, with simultaneous deposition of new micelles of the same orientation; these, although statistically perpendicular to the longitudinal axis, actually lie in a double spiral at a moderate angle on either side of that axis. However, these
conclusions are still uncertain, and a detailed discussion of the relation between growth and wall structure here would take us too far afield.

There can scarcely be any direct chemical relation between wall deposition in growth and the auxin which causes it, because the measurements and calculations of Thimann and Bonner (319) show that each auxin molecule causes the deposition of some $3 \times 10^4$ hexose residues as cellulose, as well as the pectin, hemicellulose, and protein, which also are laid down. Further, the amount of wall formed per molecule of auxin varies with temperature.

With the recognition of the other effects of auxin, the field widened. Two main viewpoints have focussed much of the research.

B. MOBILIZATION OF SPECIAL HORMONES

In brief, this view is that each process, except cell enlargement, is brought about by a specific hormone; there would be a root-forming substance, a stem-forming substance, a bud-inhibiting substance, etc. These substances are discussed in more detail in Section V of the following chapter; it is only necessary here to consider their relation to auxin. The action of auxin is visualized as causing the mobilization of these substances at the point at which the auxin accumulates. As an example, rooting of a cutting would be due to: (1) the polar transport of auxin to the base and its accumulation there, (or its direct application at the base); (2) the consequent accumulation of the root-forming hormone, "rhizocaline" at the base; and (3) action of the latter substance on the basal tissues. Similarly, swelling of the stem at the point of auxin application would be due to the mobilization by the auxin of "caulocaline" and other substances necessary for stem growth. This view has been put forward especially by Went (359; see Sect. V of Chapter III) but other authors, notably Gautheret (96), have explained their results in terms of numerous specific hormones.

Pending definite proof of the existence of such special hormones, this concept is difficult to prove or disprove. Growing loci in the plant certainly manage to accumulate water, carbohydrates, and other materials for growth, for instance in the formation of swellings. The data of Stuart (301) and Mitchell and Stewart (206), showing a marked increase of dry weight in the region where auxin is applied to a stem, are particularly clear in this connection. There is enough movement of materials to cause strong inhibition of growth above the point of application (204,284). Thus in an indirect way it must be true that auxin leads to the "mobilization" of such substances. The difficulty comes when the effect of auxin on isolated plant parts is considered. Thus, sections of coleoptile 3 mm. long, immersed in solutions of auxin and sucrose, will
grow some 100% (262). Fragments of Helianthus hypocotyl (255), or of potato tuber (123) will form roots vigorously in response to auxin. Isolated buds in solution are inhibited by auxin (272); so are isolated root tips (86, see Section VII, B). In all these instances it is difficult to ascribe any role to mobilization, yet the effect of auxin is very similar to that in the intact plant. If, however, we conclude that the evidence for the mobilization of specific hormones is insufficient, at any rate at the present time, then the alternative is that the varied effects of auxin are due to differences in the ability of different tissues to respond (314). This brings us back to the starting point and calls for a closer study of the intimate nature of the action of auxin in the cell.

C. Relation between Respiration and Growth

It has been known for a long time that growth of the coleoptile will not take place anaerobically, and Bonner in 1933 showed that growth is inhibited by cyanide, and to the same extent for a given concentration as is respiration. However, neither Bonner (33) nor van Hulssen (146) could find any acceleration of the respiration of the coleoptile by auxin alone. Hence it was concluded only that respiration is “a formal prerequisite for growth” and not that any respiratory process is involved in growth. Later work, however, has shown that the relationship is closer than that.

In the first place, cyanide is not the only inhibitor of respiration which also inhibits growth. Commoner and Thimann in 1941 found that iodoacetate is still more effective. A concentration of $2.10^{-5} M$, after a few hours delay, inhibits growth completely. This concentration, however, has little effect on respiration of the coleoptile, which requires about ten times as high a concentration for marked inhibition (Fig. 9). Since iodoacetate inhibits numerous dehydrogenases, they deduced that there is a special dehydrogenase system which takes part somehow in growth, though it cannot be responsible for more than a very small part of the respiration. Recently Bonner and Wildman (35) have made a similar discovery with respect to fluoride, namely, that low concentrations inhibit growth but do not appreciably reduce the oxygen consumption of the coleoptile. Iodoacetate and fluoride, of course, are both active on stages of the phosphorolysis cycle, and Thimann and Bonner have reported (320) that glucose-1-phosphate releases the inhibition by fluoride. From the work of James, James, and Bunting (147) it appears that the phosphorolysis cycle in plant tissue, at least in barley leaves, is similar to that in yeast or muscle, being inhibited by fluoride or iodoacetate. On the other hand, Commoner and Thimann found the iodoacetate inhibition to be reversed by malate, succinate, fumarate, and pyruvate, and
concluded that the four-carbon acid oxidation system was the one involved. This is supported by the finding of Albaum and co-workers (2,3) that intact oat seedlings are also inhibited in growth by iodoacetate and the inhibition reversed by the four-carbon acids. However, Albaum and Eichel (3) find that with intact seedlings the iodoacetate inhibition is also reversed by malonic and maleic acids, which should (in animal tissues and bacteria at least) inhibit the four-carbon acid system. Since also Berger and Avery (25) were unable to find any evidence for succinic dehydrogenase in the coleoptile, it must be concluded that at present the exact nature of the enzyme system involved in growth is not established.

![Graph](image)

**FIG. 9.**—The effect of iodoacetate on the growth (solid line) and respiration (dashed line) of *Avena* coleoptile sections. Growth may be very largely inhibited with little decrease in respiration. (From Commoner and Thimann, 70.)

One of the key enzymes is doubtless of sulfhydryl nature and its concentration appears to decrease with increasing age of the coleoptile (335a).

Very remarkable support for the conceptions of Commoner and Thimann comes from the work of Ryan, Tatum, and Giese (259) on an entirely different growth system, that of the fungus *Neurospora*. Here also iodoacetate inhibits growth while respiration is less sensitive; at about $3.10^{-3} M$, growth is reduced to zero while 30% of the respiration remains. Provided the iodoacetate concentration is not too high, the inhibition is released by succinate, fumarate, or malate, and to a lesser extent by pyruvate. The relation between growth and respiration in *Neurospora* is somewhat closer than in *Avena*, and Ryan et al. point out that inhibition of growth parallels that of respiration under certain condi-
tions, if only the iodoacetate-sensitive part of respiration is considered. Such a close parallelism does not exist in *Avena*.

Not only is respiration linked to growth, but it is also directly affected by auxin. Commoner and Thimann confirmed the older observations (see above) that coleoptile sections in water show no increased oxygen consumption when indoleacetic acid is added, but found that if the sections have been kept a few hours in sucrose there is a definite rise in respiration immediately on addition of indoleacetic acid (1–10 mg./L.). After some hours in malate the rise is larger, 20–35%. The former fact but not the increased effect of malate was confirmed by Berger *et al.* (26), who found, indeed, still larger increases due to indoleacetic acid in presence of sugar. The effect of different auxin concentrations on respiration, in presence of malate, shows a very close parallel to their effects on growth (Fig. 10). There can be little doubt, therefore, that the growth process involves a respiratory enzymic reaction as an integral part, and that auxin in some way accelerates or acts as a coenzyme for this reaction.

**D. Relation Between Growth and Protoplastmic Streaming**

In his fundamental experiments on auxin, Went (348) noted the speed of protoplastic streaming in the coleoptile and suggested that it might be responsible for auxin transport. While this has been neither confirmed nor disproved, it has become increasingly probable that streaming is connected with the growth process and the effect of auxin. In studying the effect of light on growth, Bottelier (38,39) discovered some remarkable
parallels between streaming and growth. Exposure to light temporarily retards the rate of streaming as also the rate of growth, and the proportion between the effectiveness of different wavelengths is the same for streaming as for growth. Further, both streaming and growth show a similar dependence upon oxygen, which varies with age of the coleoptile. This was shown indirectly by following the effect of temperature on streaming rate (39). The rate increases with temperature according to the usual van't Hoff relationship but flattens off at about 21° in young (96-hour) coleoptiles; this flattening can be prevented by saturating the water with oxygen. Old (260-hour) coleoptiles show no such flattening of the curve, which continues upward to 33°. Even in old coleoptiles the curve can, however, be flattened by bubbling nitrogen through the water. The rate at which oxygen is consumed for streaming therefore decreases with increasing coleoptile age.

This fact was confirmed by Thimann and Sweeney, who subsequently made an extensive study of the effect of auxin on protoplasmic streaming in the coleoptile. They first found (334) that auxin in physiological concentrations produces a temporary acceleration of the streaming rate, which returns to normal after about twenty minutes. If, however, sugar is added, the acceleration is maintained for several hours (304), as is the growth rate (see Fig. 11 A). The acceleration is dependent on the access to oxygen; it cannot be obtained after infiltration of the intercellular spaces with water (224,302), nor during treatment with dinitrophenol (334), which presumably increases the rate of oxygen consumption and thus lowers the oxygen tension in the solution. Further analysis (305) showed that, when the conditions are such that auxin alone will not accelerate the streaming, simultaneous treatment with auxin and malate produced a maximal acceleration. These conditions include (a) very dilute auxin (indoleacetic acid 0.001 mg./l.), (b) coleoptiles too old (6 days old), and (c) coleoptiles cut off and soaked 24 hours in water or fructose solution (Fig. 11 B). Finally, the acceleration is prevented by iodoacetate in the same concentration as it prevents growth, namely, $5 \times 10^{-5} \text{M}$, and this inhibition is reversed by malate. The data thus indicate that the basal streaming rate is not influenced by auxin; auxin, however, accelerates the rate through influencing an oxidative reaction involving sugar and malate, which is most probably the same reaction as that which leads to growth. It is interesting to note that in old coleoptiles, in which elongation cannot occur because secondary wall has been laid down, the typical acceleration of streaming by auxin and malate may still take place. In other words, the fundamental (enzymic) growth process need not necessarily cause visible growth (see 305,317). Since the streaming acceleration occurs before any detectable growth accelera-
tion, it may well be the cause of the accelerated growth. It is possible, too, that the acceleration of streaming is the means whereby accelerated accumulation of plastic materials for the growth process (see pp. 52, 57) is brought about.

As shown in Section VII, B, the growth of roots is inhibited by all
but excessively low concentrations of auxin. It is of interest that Sweeney (303) finds that the rate of streaming in root hairs of *Avena* is accelerated by much lower auxin concentrations than in the coleoptile, the optimum concentration being $10^{-4}$ mg./l. as against about 0.1 mg./l. in the coleoptile cells. Inhibition of streaming also takes place at somewhat lower concentrations than in the coleoptile, but, curiously enough, removal of the seed and coleoptile seems to reduce the sensitivity of the root hairs to high auxin concentrations. Sweeney also found that streaming continues at the normal rate in fully plasmolyzed root hairs, thus making it unlikely that streaming has its inception at the protoplasm–cell wall interface.

The way in which the streaming rate could be affected by auxin is, of course, unknown. Northen (222) has found that treatment with auxin decreases the viscosity of protoplasm, and that this effect parallels, at least roughly, the effects on growth. While a reduction in viscosity would doubtless lead to an increase in the rate of flow, the causal connection, if any, will need to be established by studying both phenomena on the same material. Probably both are related to the respiratory effects described above.

**E. Growth and Uptake of Water**

In its simplest form, the enlargement of plant tissues can be considered as depending on uptake of water. This must of course be accompanied or followed by synthesis of protoplasm and of cell wall. Since isolated sections of stems or coleoptiles will, however, grow 100% or more in sugar and auxin alone, nitrogen uptake and protein synthesis evidently is not an integral part of the primary growth process. The experiments of Reinders with slices of potato and other materials are therefore of considerable interest because, instead of measuring elongation, Reinders (250,251) measured increase in weight in water (or auxin solution), which is a direct measure of water uptake. In general, her results are like those with coleoptile sections in that auxin (especially indoleacetic acid, 1 mg./l.) strongly promotes water uptake in a strictly aerobic process. Dry-weight losses indicate that the auxin also stimulates respiration in this material, particularly in the later stages of an experiment lasting several days. If auxin exerts its effect directly in increasing the plasticity of the cell walls, as in the view of Heyn and Söding, then the increased water uptake would be accounted for at once on osmotic grounds. This, however, appears not to be the case. Thimann and Schneider (326) showed that low concentrations of potassium chloride considerably promote growth in auxin solution, and that growth of coleoptile sections is a linear function
of the osmotic gradient. This last point was established by using mannitol, to which plant cells are highly impermeable (65,66), in the external solution; van Overbeek (cited in 326) has reported similar results with sucrose. Commoner, Fogel, and Müller (68) have shown that the water intake can occur against an osmotic gradient, i.e., in presence of sucrose solution of plasmolyzing concentration. Conductivity measurements (Commoner and Mazia, 69, and unpublished data)\(^{10}\) show that the potassium chloride, as well as the water, is taken into the tissue against the osmotic gradient. Commoner \textit{et al.} also showed that this water uptake is inhibited by iodoacetate. It is, however, true that growing tissues show no change in their osmotic pressure, as against nongrowing ones, particularly when in auxin without sugar (234), so that the water and electrolyte must be taken in strictly parallel with growth, and perhaps the osmotic pressure may equally be maintained internally by starch hydrolysis. Indeed, auxin does promote starch hydrolysis (208). It is tempting to consider the salt uptake to be the primary process, for, as Commoner (67) points out, salt uptake is, like growth, well known to be typically an aerobic process, requiring carbohydrate and associated with active protoplasmic streaming (140,295,296). On the other hand, starch-rich tissues like potato grow to a considerable extent in distilled water, as shown by Reinders (251), so that uptake of externally applied salts is not necessarily a feature of primary (short-term) growth. Further analyses of these relationships will undoubtedly shed important light on the fundamental nature of growth.

**F. Conclusions**

The general concept of auxin action which emerges from the facts presented can be summarized as follows:

The auxin may produce a variety of different effects, depending on: (a) its concentration, (b) the tissues on which it acts, (c) its stability in these tissues, and (d) the relative ease with which it is transported in the plant. These different effects in all probability spring from one fundamental master reaction in the cell.

The structural requirements for auxin action point to the need for a particular set of polar groupings in a particular spatial array, \textit{i.e.}, they suggest that the molecule has to combine with a determined spatial structure.

There is abundant evidence that auxins combine with proteins, and though the exact nature of the combination is obscure, it is probable that

\(^{10}\) The author desires to thank Dr. Commoner for making available unpublished data and discussion.
different types of combination may occur, and certain that many different proteins are involved.

The auxins act catalytically.

The action involves a respiratory process which concerns carbohydrate and the organic acids; this process is linked directly with the protoplasmic streaming.

If we put these simplified conclusions together, it is evident that they point in one direction: auxin is a coenzyme (or prosthetic group) for some fundamental enzymic process in the cell. This process is a bottleneck, or limiting factor, through which the uptake of solutes and/or water, the deposition of cellulose, and all the other appurtenances of growth must flow. Which process is the primary one, if any, and which are secondary remains unsolved.

REFERENCES

PLANT GROWTH HORMONES

177. Kuhn, R., Jerchel, D., Moewus, F., Müller, E. F., and Lettré, H. Naturwissenschaften 31, 468 (1943).
185. Langham, D. G. ibid. 28, 551–566 (1941).
187. Larsen, P. 3-Indole-acetaldehyde as a growth hormone in higher plants. Diss., Copenhagen (1944).
188. La Rue, C. D. Am. J. Botany 22, 903 (1935).
189. La Rue, C. D. ibid. 23, 520–524 (1936).
196. Link, G. K. K., and Eggers, V. ibid. 103, 87–106 (1941).
225. van Overbeek, J. *ibid.* 30, 537-626 (1933).
228. van Overbeek, J. *ibid.* 100, 133-166 (1938a).
229. van Overbeek, J. *J. Heredity* 29, 339-341 (1938b).
234. van Overbeek, J. *ibid.* 51, 265-269 (1944).
PLANT GROWTH HORMONES

266. Schuringa, G. J. Diss., Utrecht (1941).
320. Thimann, K. V., and Bonner, W. D., Jr. Unpublished data reported to AAAS meeting, Boston (1940), and *Am. J. Botany in press* (1948).
351. Went, F. W. *ibid.* 37, 547–555 (1934b).
Addendum

Since writing this chapter on plant growth hormones a large number of papers have been published dealing with problems discussed in the text. These have raised several new points but on the whole it is felt that the conclusions reached have not suffered any important changes in principle. In particular the mode of action of auxin still remains uncertain, the problems concerned with the formation of gall and tumor tissues have not yet been cleared up and the mechanism of auxin redistribution in the tropisms remains as obscure as ever. The biogenesis of indole-acetic acid seems somewhat strengthened and many additions have been made in the technical uses of the auxins.

For these reasons it has been thought hardly worth while to rewrite the text, but the papers which have appeared during the last two and a half years are listed as a supplementary bibliography at the end. The subdivision of this list into the section headings of the chapter should help to make the list useful for reference. For additional guidance, a word or two as to the specific subject matter of each paper has been inserted. It is not claimed that the listing is complete but it is believed that the majority of papers of importance are included.

SUPPLEMENTARY REFERENCES

I. GENERAL REVIEWS

II. ASSAY METHODS
C. Straight Growth Measurements
PLANT GROWTH HORMONES


F. Other Methods


Nysterakis, F. Compt. rend. 229, 527-529 (1949).

III. CHEMISTRY OF AUXINS

A. "Auxin a and b"


B. Indole-3-acetic Acid

Gautheret, R. J., and Raoul, Y. Bull. soc. chim. biol. 31, 1635-1638 (1949); indoleacrylic acid.


Kramer, M., and Went, F. W. Plant Physiol. 24, 207-221 (1949); IAA in tomatoes.

Larsen, P. Am. J. Botany 36, 32-41 (1949); inactivation of indoleacetaldehyde.

Tang, Y. W., and Bonner, J. ibid. 35, 570-578 (1948); inactivation of IAA.

Wagenknecht, A. C., and Burris, R. H. Arch. Biochem. 25; 30-53 (1950); inactivation of IAA.


C. Synthetic Substances Not Known to Occur Naturally

Bertossi, F. Compt. rend. 231, 161-163 (1950); diphenylylacetic.


Hoffmann, O. L., and Smith, A. E. Science 109, 588 (1949); N-aryl-phthalamic acids.


Schocken, V. Arch. Biochem. 23, 198-204 (1949); tryptophane.

IV. TRANSPORT OF AUXIN

A. Polar Transport and Its Mechanism


B. Upward Transport

Corns, W. G.  *Can. J. Research* C26, 239–248 (1948); 2,4-D transport.


Rohrbaugh, L. M., and Rice, E. L.  *ibid.* 110, 85–89 (1949); sugar and 2,4-D transport.

V. ROLE OF AUXIN IN TROPISMS

A. Geotropism


B. Phototropism


Galston, A. W., and Baker, R. S.  *Am. J. Botany* 36, 773–780 (1949); role of riboflavin.

Galston, A. W., and Hand, M. E.  *ibid.* 36, 85–94 (1949); light inhibition of growth.


C. Other Tropisms


VI. ROOT FORMATION

B. Substances Active


Murray, M. A., and Whiting, A. G.  *Botan. Gaz.* 110, 404–426 (1949); 2,4-D and corn.

See also next chapter, Section V, A

C. Interactions between Factors


D. Anatomical Studies


E. Methods of Treatment


Naundorf, G.  *Notas Agronomicas (Palmira, Colombia)* 3, 97–101 (1950); coffee.

VII. PHENOMENA OF INHIBITION AND TOXICITY

General

Naundorf, G.  *Notas Agronomicas (Palmira, Colombia)* 3, 1–61 (1950); review of inhibitions, 343 refs.

A. Bud Inhibition


Champagnat, P.  *Rev. gén. botan.* 56, 333–351 (1949); early laterals.
PLANT GROWTH HORMONES

Leopold, A. C. *Am. J. Botany* 36, 437-440 (1949); tillering in barley.

2. MECHANISMS
Galston, A. W. *Plant Physiol.* 24, 577-586 (1949); interaction with nicotinic acid.
Hemberg, T. *Physiol. Plantarum* 2, 37-44 (1949); inhibitors in Fraxinus buds.

3. GENERAL SIGNIFICANCE
Hemberg, T. *Physiol. Plantarum* 1, 24-36 (1949); potato rest period.
Steinberg, R. A. *Plant Physiol.* 25, 103-113 (1950); tobacco.

B. Root Inhibition
Åberg, B. *Physiol. Plantarum* 3, 447-461 (1950); auxin antagonists and synergists.
Audus, L. J. *New Phytologist* 47, 196-219 (1948); reversibility.
Audus, L. J. *ibid.* 48, 97-114 (1949); pH effect, 2,4-D.
Burström, H. *Physiol. Plantarum* 3, 277 (1950); acceleration, “anti-auxins.”
Lundgårdh, H. * Arkiv Botan. 1*, 295-299 (1949); bleeding.
Moewus, F. *Der Züchter* 19, 108-115 (1948); assay, applications.
Slankis, V. *Physiol. Plantarum* 1, 390-392 (1948); 3, 40-44 (1950); relation to mycorrhiza.
Slankis, V. *Svensk. Botan. Tidskr.* 43, 603 (1949); relation to mycorrhiza.
Wilske, C., and Burström, H. *Physiol. Plantarum* 3, 58-67 (1950); thiphenoxyacetic acids.

See also Section IX A

C. Inhibition of Abscission

D. General Toxicity
Hitchcock, A. E., and Zimmermann, P. W. *Contribs. Boyce Thompson Inst.* 15, 173-194 (1948); adjuvants and 2,4-D.
Spear, I., and Thimann, K. V. *Plant Physiol.* 24, 587-600 (1949); onion juice and 2,4-D.

VIII. OTHER ACTIONS OF AUXIN
A. Cell Division

1. TISSUE CULTURES
Camus, G. *Rev. cytol. biol. vég.* 11, 1-199 (1949); general.
Duhamet, L. *Compt. rend.* 229, 1353-1354 (1949); 230, 770-771 (1950); coconut milk.
Duhamet, L. *Compt. rend. soc. biol.* **144**, 59–61 (1950); coconut milk; stem cultures.
Gautheret, R. J. *ibid.* **144**, 172–173, 622–626 (1950); cambium cultures, growth factors.
Goris, A. *Compt. rend.* **231**, 870–872 (1950); coconut milk; sugar consumption.
Kulescha, Z. *Compt. rend. soc. biol.* **143**, 1449–1450 (1949); *ibid.* **144**, 179–181 (1950); auxin in tissue cultures.

2. CAMBIUM

Camus, G. *Compt. rend. soc. biol.* **141**, 38–40 (1947); *Rev. cytol. biol. vég.* **1**, 1–199 (1949); morphogenesis, substances compared, etc.

3. OTHER TISSUES


4. PATHOLOGICAL CHANGES

White, P. R. *Quar. Rev. Biol.* **26**, 1–16 (1951); tumor tissue.

B. Formation of Fruits

Moewus, F. *Planta* **37**, 413–430 (1949); auxin in fruits.
Stewart, W. S., and Condit, I. J. *ibid.* **36**, 332–335 (1949); fig.
Swanson, C. P., La Velle, G., and Goodgal, A. H. *ibid.* **36**, 170–175 (1949); Tradescantia.
PLANT GROWTH HORMONES

Wain, R. L.  *J. Hort. Sci.* **25**, 249-263 (1950); tomato.
Weaver, R. J., and Williams, W. O.  *Botan. Gaz.* **111**, 477-485 (1950); grapes.

**C. Role of Auxin in Seed Germination**

Naundorf, G., Villamil, G. F., and Medina, J.  *Notas Agronomicas* (Palmira, Colombia) **3**, 63-88 (1950); cacao, auxins and inhibitors.

**IX. MECHANISM OF ACTION**

**General**


**A. Effects on Cell Wall**

Lundegärdh, H.  *Arkiv Botan.* **1**, 289-293 (1949); roots.

**B. Mobilization of Special Hormones**


**C. Relation between Respiration and Growth**

Bonner, J.  *Arch. Biochem.* **17**, 311-325 (1948); coleoptile respiration.
Christiansen, G. S.  *Arch. Biochem.* **29**, 354-368 (1950); exudate from pea stems.
Kelly, S., and Avery, G. S.  *Am. J. Botany* **36**, 421-426 (1949); 2,4-D.
Raadt, E.  *Planta* **36**, 103-130 (1948); auxin and ascorbic acid.
Smith, F. G.  *Plant Physiol.* **23**, 70-83 (1948); 2,4-D.
Taylor, D. L.  *Botan. Gaz.* **109**, 162-176 (1948); 2,4-D.

**E. Growth and Uptake of Water**

Levitt, J. *Plant Physiol.* **23**, 505–515 (1948); potato sections.

**Miscellaneous**

Corn, W. G. *Can. J. Research* C28, 393–405, (1950); 2,4-D.
Freeland, R. O. *Botan. Gaz.* **111**, 319–324 (1950); effects on respiration and photosynthesis.
Gall, H. J. F. *ibid.* **110**, 319–323 (1948); starch digestion.
Sivori, E. M., and Claver, F. K. *Rev. argentina agron.* 17, 1–10 (1950); action of 2,4-D upon enzymes.
Struckmeyer, B. E. *Botan. Gaz.* **111**, 130–139 (1949); interaction with Ca.

**Applications to Horticulture**